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Risk factors, pathologies and control of gut-dwelling roundworms in chickens

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Summary

Nematode infections pose a threat to backyard chickens, presenting serious health and productivity challenges. The study aimed to assess risk factors related to pathological changes and the effect of mebendazole and its derivatives against nematode infections in domestic chickens.
A total of 456 chicken faecal samples were randomly collected and analysed using the direct smear, flotation methods and sedimentation method, and histopathological analysis was performed. Sixty infected chickens were divided into three groups: X (control), Y (mebendazole), and Z (derivative), treated orally at 10 mg/kg for three days. Data were analyzed in R (v2025) to compute percentages, 95 % CIs, p-values, and odds ratios.
Overall, 72 % (330/456) of chickens were infected, with nematode parasite, *Ascaridia galli,* being the most prevalent species, 38.5 % (127/ 330). Single infections were the most common 70.3 % (232/330), followed by double infections 24.5 % (81/330), and triple infections 5.2 % (17/330). Infections were more common during the wet season 65 % (215/330) and were higher in females 74.6 % (200/267), young 85 % (233/274), and weak chickens 90.6 % (126/139), free-range systems 74.3 % (255/355) and non-dewormed chickens 73.3 % (262/356). Multivariate logistic analysis showed significant associations ($p < 0.05$) with age, gender body condition, and management type, Histopathological alterations revealed hemorrhagic enteritis, necrotic patches, intestinal inflammation, submucosal oedema, glandular hyperplasia and villous atrophy. Anthelmintic trials demonstrated that both Mebendazole and its derivative significantly reduced the eggs per gram (EPG) of faecal samples of roundworm, with the derivative being more effective.
Nematode infections cause significant health problems and economic losses in poultry; therefore, improved management, sanitation, and regular deworming practices are essential to effectively control parasitic infections in domestic chickens.
Keywords: Roundworms; Parasitic pathogens; scavenging chicken; pathology; treatment

Introduction

Poultry farming related to domestic chicken is known to be one of the most important sectors of agriculture (Nanda *et al.*, 2022). According to the Food and Agriculture Organisation (FAO), the global poultry population is around 14 billion, approximately 75 % situated in the developing world (Ara *et al.*, 2021). Traditional free-range poultry farming plays a vital role in the rural economies of poor communities (Berhe *et al.*, 2019). It provides a crucial source of protein (El-Debakhy *et al.*, 2024). Additionally, it is a source of

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income for poor communities (da Silva *et al*., 2022). Chicken meat and eggs are vital for nutrition in poor families (Ameji *et al.*, 2022). Sales in local markets provide cash income (Islam *et al*., 2020). Scavenging chickens are hindered by malnutrition practices. Poor body condition and inadequate management systems. In the backyard production system (Idika *et al*., 2016). Free-range chickens are at higher risk of infection by a wide variety of parasites (Malik *et al*., 2022), lice, mites, fleas, ticks, and helminths (Saemi Soudkolaei *et al*.,2021). Coprological feeding of chickens is a routine practice that leads to infection of the chicken with a large number of helminth parasite species (Tay *et al*., 2017). Common chicken nematodes include *Ascaridia galli*, *Heterakis gallinarum*, *Capillaria* spp., and *Trichuris* spp., targeting different parts of the gastrointestinal tract (Ara *et al*., 2021).

A. galli has a direct life cycle; eggs develop into second-stage larvae (L2) in 8 – 21 days, depending on temperature (Hoglund *et al*., 2023). Chickens ingest embryonated *Ascaridia* eggs; L3 larvae hatch, migrate into and out of the mucosa, then mature in the intestinal lumen (Shohana *et al.*, 2023). *H. gallinarum* adults inhabit the cecum; eggs passed in faeces become infective and are ingested directly or via earthworms, which transport them. The prepatent period is 5 – 6 weeks (Rahimian *et al.*, 2016). Once ingested, larvae hatch, migrate to the cecum, and mature into adults, completing the cycle (Thomas *et al.*, 2024). *Capillaria* species, also called hairworms, are thin gastrointestinal parasites of poultry, affecting either the upper (crop, oesophagus) or lower (intestine, ceca) digestive tract (Yousaf *et al*., 2019). They have direct or indirect lifecycles (Bajoi *et al*., 2024). In the direct life cycle, chickens ingest *Capillaria* embryonated (infective) eggs, which develop into adults in the intestine without tissue migration (Sarba *et al.*, 2020). Indirect-cycle species like *C. caudinflata*, *C. bursata*, and *C. anuulata* require earthworms; chickens ingest them, with a period of about three weeks, causing gut damage (Jilo *et al.*, 2022). In chickens, *Trichuris* infection occurs from ingesting embryonated eggs, causing intestinal damage, inflammation, and ulceration, leading to diarrhoea, anaemia, weight loss, and possible death (Filbey *et al*., 2019). Gastrointestinal nematode parasites in backyard chickens result in reduced egg production (Sadeghi *et al*., 2024). It also causes diarrhoea, intestinal obstruction, morbidity hemoglobin depression, and heightened mortality rates. High death rate, especially among the young, if remains untreated (Shifaw *et al*., 2021). Anthelmintic vaccines for poultry helminths are not yet developed (Shirin *et al*., 2025).

Control mainly relies on prophylactic drug therapy with albendazole, fenbendazole, Mebendazole, piperazine, levamisole, and Ivermectin. Benzimidazole and piperazine resistance in *Ascaridia galli* was recently detected by Ritu *et al*. (2024). There is limited data on the efficacy of commercial anthelmintic against nematodes, and the worm's pathological impact remains understudied. This study aims to assess infection prevalence, characterize induced pathologies, and evaluate the efficacy of Mebendazole and its derivative against nematode infection.

Materials and Method

Study Area

The present study was conducted in three districts of the Malakand region, namely District Lower Dir (34.9161°N, 71.8097°E), District Swat (35.2220°N, 72.4258°E), and District Malakand (34.5656°N, 71.9304°E) are located in northern Khyber Pakhtunkhwa, Pakistan (Fig. 1). The region experiences a temperate climate characterised by cold winters and hot, humid summers due to monsoon rains in warm months of the year (June – September). The humid climate, with high rainfall, favourable conditions for the transmission of parasitic infections in domestic livestock and poultry.

Sample Size

The sample size was computed from the formula (Daniel & Cross, 2019), $n = Z^2P(1 - P) / d^2$ with the confidence at 95 % and expected prevalence at 50 % and precision at 0.05, which we used to produce 385. We actually conducted the survey in 456 domestic chickens.

Duration of Study

The present work was conducted from January through December 2023. In the meanwhile, faecal samples were collected and chickens were obtained for chemotherapy and histopathological studies. Data pertaining to potential risk factors including age, gender, health status, and management practices were also obtained from the caretaker owners via a structured questionnaire.

Study season

The seasonal differences were determined from the year's partitioning into two parts with respect to rainfall: the dry season (from March till mid-June and mid-September till February), covering the spring, summer preceding the monsoon, autumn, and winter; and the rainy season (from late June till mid-September), i.e., the monsoon season.

Sample collection and preservation

3 – 5 g fresh faeces were collected immediately from the birds' cloaca in labeled bottles with 2.5 % potassium dichromate. Kept in sealed sterile containers at 4 – 8°C in insulated ice-packs from the field site and then stored at 4°C in the laboratory of University of Malakand for further analysis.

Laboratory Analysis

Direct wet smear and concentration techniques were used to detect nematode eggs in fecal material. Briefly, one gram of faeces was mixed with 20 ml of sodium chloride (NaCl) solution and thoroughly filtered. The resulting filtrate was transferred into a test tube until a meniscus was formed at the top. A coverslip was gently placed over and left for 12 to 20 minutes. After that, the coverslip was carefully removed and examined under a light microscope for the presence of nematode eggs.

Determination of changes in the intestine

During post-mortem, parasitically damaged suspected tissues were collected and preserved in Carnoy's solution (3:1 ethanol to acetic acid) for 48 hours with shaking. Samples were washed with PBS (phosphate buffer solution), dehydrated, cleared, and embedded in paraffin. Thin 5 µm sections were stained with H&E and examined under the light microscope (10x and 40x, Olympus DP71). Three sections and three foci per sample were analysed.

Effect of the Anthelmintic Drugs

Sixty domestic 30-week-old chickens already naturally infected with nematode parasites, tagged, housed separately, and provided with clean water and grain ad libitum for seven days without parasitic treatment. Based on their EPG (eggs per gram) values, domestic chickens were randomly allocated into groups X, Y, and Z, each with three replicates of five chickens, following the experimental design. Group X was the untreated control, Group Y received Mebendazole at 10 mg per 1.5 kg body weight, and Group Z was administered an oral Mebendazole derivative at the same dosage for three consecutive days. Faecal samples were collected on days 7, 14, and 21 post-treatments for egg reduction analysis. Drug efficacy was calculated using the formula of Soulsby (1982)

$$\% = \frac{\textit{Mean pre treatment EPG - Post treatment EPG}}{\textit{Mean pre treatment EPG}} \times 100$$

Statistical Analysis

The obtained data were put in Microsoft Excel to determine the statistical variation in different factors. The computations were conducted with the using of R software for multivariate regression test. Anthelmintic efficacy was evaluated using one-way ANOVA, followed by Tukey's post-hoc test.

Ethical Approval and/or Informed Consent

The study protocol was approved by the Institutional Bioethics Committee University of Malakand (Ref: No: UOM/Admin/2025/1325).

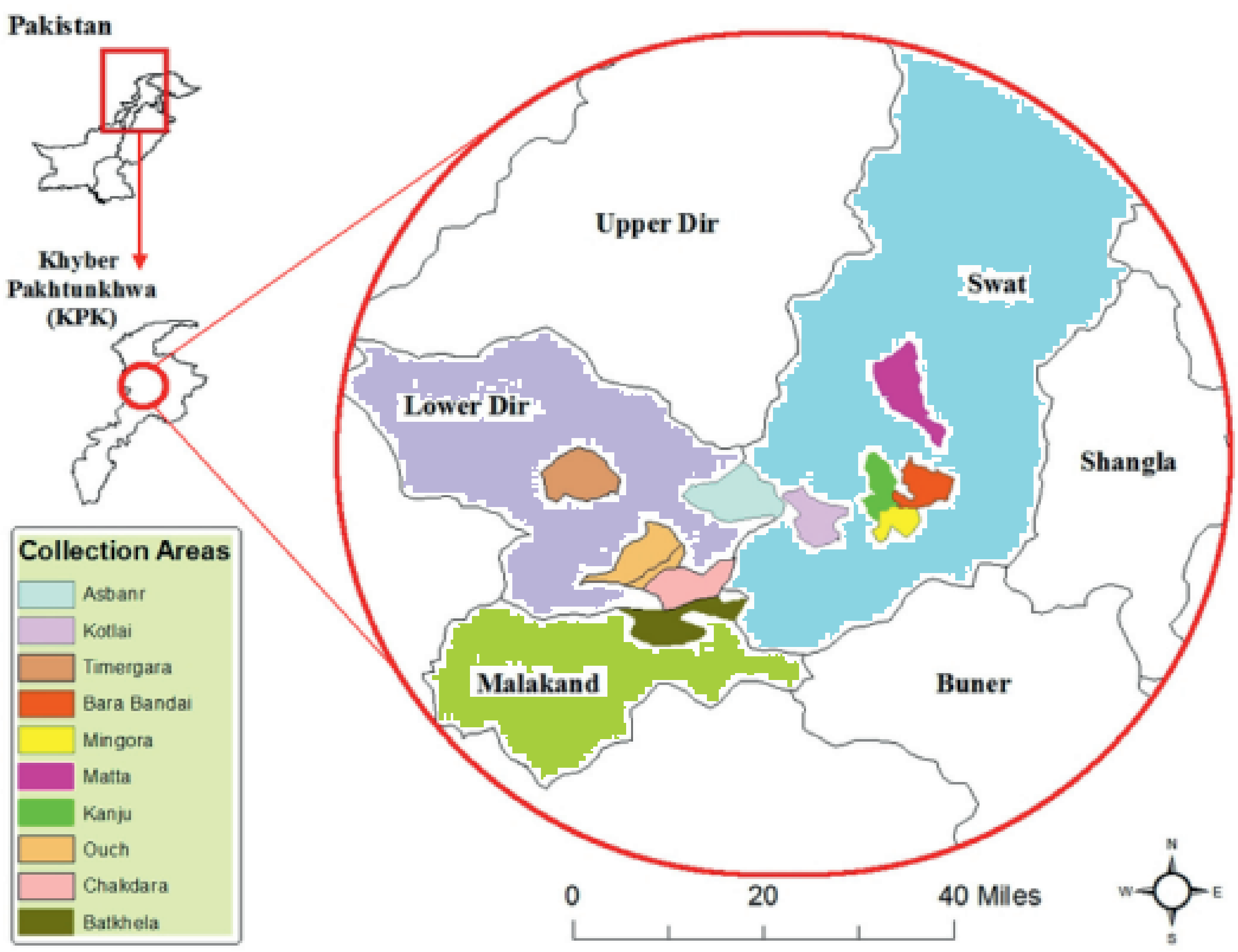

Fig. 1. Map of Malakand Division showing sample collection areas (self-generated via ArcGIS)

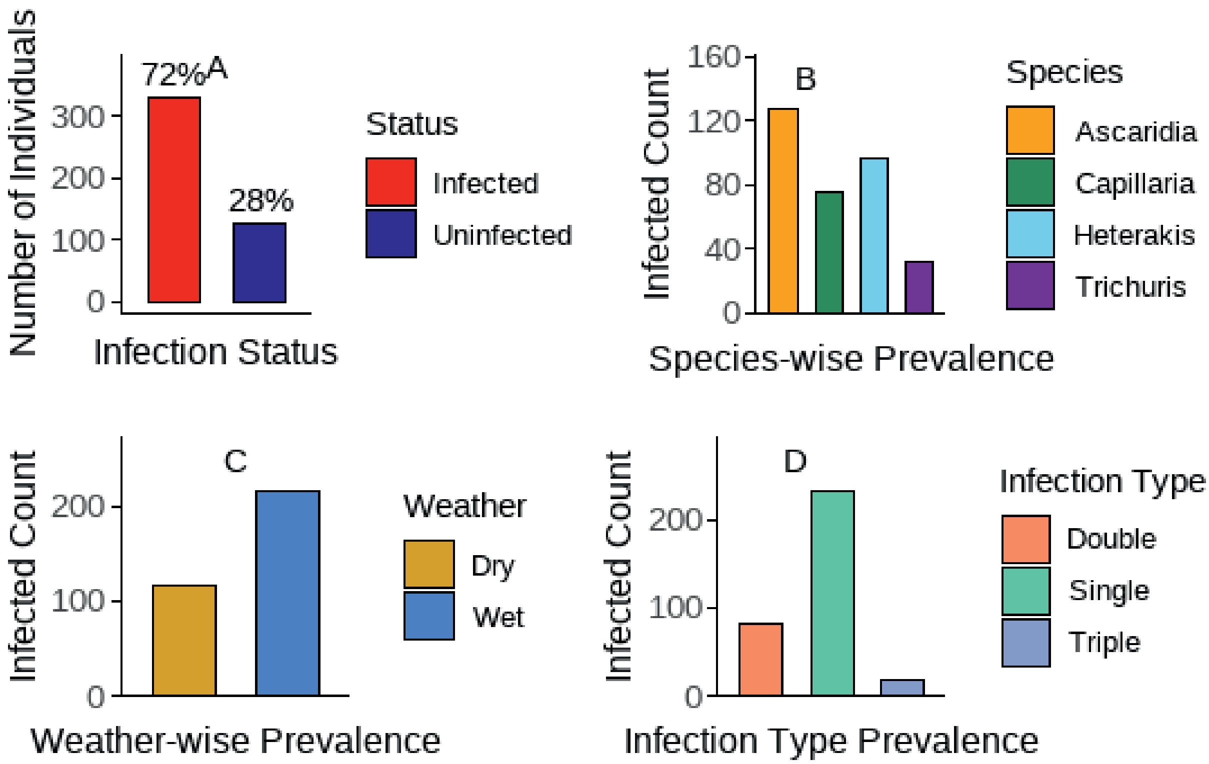

Fig. 2. Prevalence of nematode infection in chickens: Overall infection (A). species-wise prevalence (B); infection types (C); weather-wise infection (D).

Results

Prevalence, Species Distribution, and Seasonal Variation

Overall, an infection rate was detected in 72 % (n=330/456) of the examined chickens found to be infected with one or more species of nematode (Fig. 2A). Regarding species, *A. galli* was the most prevalent parasite found in 38.5 % (n=127/ 330) (Fig. 2B). Infection types showed a high prevalence of single infections, accounting for 70.3 % (n=232/330), followed by double infections 24.5 % (n=81/330), and triple infections 5.2 % (n=17/330) (Fig. 2C). The chickens screened in the wet season were noted with the highest prevalence 65 % (n=215/ 330) cases, as compared to the dry season, 34 % (n=115/ 330) (Fig. 2D).

Multivariate Logistic Regression Analysis of Associated Risk Factors in Chickens

Table 1. presents the results of the multivariate logistic regression analysis conducted to evaluate the association between various host and management-related risk factors and the prevalence of gastrointestinal nematode infections in domestic chickens. The highest prevalence of gastrointestinal nematodes was recorded in female chickens (74.6 %) compared to males (68.8 %), statistically significant with (OR = 1.47, 95 % CI = 1.01 – 2.17, *P* = 0.044). Young chickens showed a particularly high prevalence at 85 % as compared to adults 53 % statistically significant with (OR = 0.25, 95 % CI = 0.16 – 0.41, *P* = 0.001). Weak chickens had the highest prevalence rate (90.6 %) compared to healthy 64.4 % statistically significant with (*OR* = 3.85, 95 % CI = 2.23 – 6.63, *P* = 0.001). Free-range rearing also showed a higher prevalence (74.3 %) compared to semi-scavenging systems (71.8 %), statistically significant with (*OR* = 1.54, 95 % CI = 1.02 – 2.32, *P* = 0.039). Regarding feeding, both scavenging (74.8 %) and additional feeding (71 .1 %) were observed (OR = 1.41, 95 % CI = 1.00 – 2.29, *P* = 0.047). Non-dewormed chickens had a higher prevalence (73.3 %) compared to dewormed (69 %), with statistically significant (*OR* = 1.61, 95 % CI = 1.03 – 2.63, *P* = 0.036). Locality-wise, infection was highest in Malakand (75.5 %), followed by district Lower Dir (72 %)and district Swat (69.1 %). The odds of infection were significantly higher in Malakand (OR = 1.49, 95 % CI = 1.02 – 2.18, *P* = 0.041), while the difference in Lower Dir was not statistically significant (OR = 1.32, 95 % CI = 0.87 – 1.99, P = 0.18).

Intestinal Alteration Caused by Nematodes in Chickens

Pathological changes revealed inflammation, reddish hemorrhagic spots on the intestinal wall, acute fibrous enteritis with clotted blood, necrotic patches, and consolidation of intestinal contents, mainly in savior infected birds. A thorough visual examination was conducted on both infected and uninfected small intestines (Fig. 3a). Pathological changes observed in the intestine of domestic chickens revealed submucosal oedema with minimal cellular infiltration,

Table 1. Multivariate Logistic Regression Analysis of Associated Risk Factors in Chickens (n= 456).

Variable	Category	Examined (n)	Infected (n)	Prevalence (%)	OR	95% CI	*P*-value
Gender	Male	189	130	68.6	Ref	—	—
	Female	267	200	74.8	1.47	1.01 – 2.17	0.044*
Age	Young	274	233	85	Ref	—	—
	Old	182	97	53.3	0.25	0.16 – 0.41	0.001***
Body condition	Healthy	317	204	64.4	Ref	—	—
	Weak	139	126	90.6	3.85	2.23 – 6.63	0.001***
Rearing System	Semi-intensive	101	75	71.8	Ref	—	—
	Free range	355	255	74.3	1.54	1.02 – 2.32	0.039*
Feeding Type	Additional feed	112	83	71.1	Ref	—	—
	Scavenging	344	247	74.8	1.41	1.00 – 2.29	0.047*
Deworming Status	Yes	100	69	69	Ref	—	—
	No	356	261	73.3	1.61	1.03 – 2.63	0.036*
Locality	Swat	152	105	69.1	Ref	—	—
	Malakand	151	114	75.5	1.49	1.02 – 2.18	0.041*
	Lower Dir	153	111	72.5	1.32	0.87 – 1.99	0.18

n= number, OR = odd ratio, CI = Confidence interval, **P* < 0.05 (statistically significant), ****P* < 0.001 (very highly significant)

accompanied by hyperplasia of the elongated submucosal glands. There is a focal accumulation of inflammatory cells (FACI) due to dead necrotic debris from adjacent glandular tissue in the submucosa layer in the small intestine (Fig. 3 b).

The submucosal glands of the small intestine in domestic chicken exhibited marked proliferation along with some degree of atrophy of the intestinal villi (Fig. 3c).

Severe necrosis of the intestinal villous cells, along with the shedding of epithelial cells and hyperplastic crypt structures, was noticed (Fig. 3d).

Mebendazole and its derivatives in the control of nematode infection

The mean EPG (eggs per gram) counts for *A. galli*, *H. gallinarum*, *Capillaria* spp., and *Trichuris* spp. were evaluated across the treatment groups: Control, Mebendazole, and a Derivative compound. EPG counts in the Control group rose with time, with the maximum in *A. galli* at 370, confirming the continuance of infection. Mebendazole was moderately effective in decreasing EPGs by 70 – 78 % with post-treatment EPGs in the range 55 – 75 % in all species. Derivative compound proved the most efficacious with EPG reductions of 88 – 92 % and with EPGs decreasing lowest at the level of 17.6 in *Trichuris* spp. and 28 – 30 % in other groups at day 21. Statistics verified the result that the derivative compound treatment proved more efficacious against the nematode ($P < 0.0001$) in comparison with the mebendazole-treated group. The present result suggests that while Mebendazole does possess moderate anthelmintic activity, the Derivative compound is substantially more effective in reducing gastrointestinal nematode burdens in chickens (Table 2).

Egg reduction rate

The egg per gram (EPG) count reduction after treatment was calculated. A higher egg negative rate (%ENR) was observed with the Derivative treatment compared to Mebendazole across all tested nematode infections. Derivatives achieved ENR values ranging from 87.5 % to 92.2 %, with the highest efficacy observed against *Trichuris* spp. (92.2 %), followed by *A. galli* (90 %), *Capillaria* spp. (90.6 %), and *H. gallinarum* (87.5 %), as compared to Mebendazole, with %ENR values ranging from 70.3 % to 78.1 %. Chi-square analysis showed that the derivative had a consistently higher egg reduction rate than mebendazole; the differences were not statistically significant ($p > 0.05$) (Table 3).

Discussion

Current investigation highlights the risk factors associated with pathological alterations and control of nematode infections in domestic chickens. The current findings show 72 % prevalence, which is in accordance with the findings reported by Atique *et al.* (2025) from Gujranwala, Punjab, Pakistan, Hasan *et al*. (2025) from Bangladesh and Ara *et al.* (2021) from Kashmir, India. Even higher rates have been reported in free-roaming chickens in Africa (Asumang *et al*., 2019). The variations of these findings highlight the significant risk of nematode infection in low-input, rural poultry systems.

In the present study, *A. galli* was the most prevalent parasite, which was consistent with the findings of Atique *et al.* (2025) from Punjab, Pakistan; Ara *et al*. (2021) from India; Hasan *et al.* (2025) from Bangladesh and Temesgen *et al.* (2024) from Ethiopia, revealing

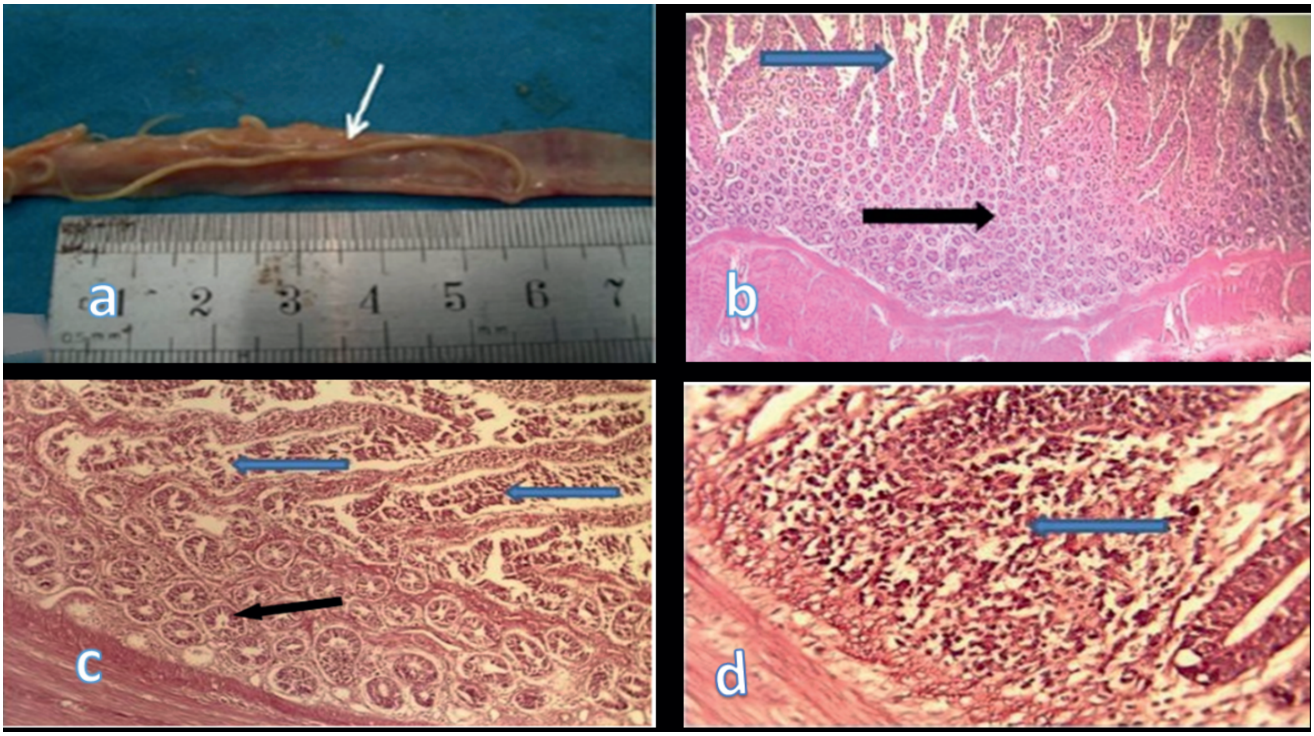

Fig. 3. Histopathological changes in small intestine: Gross pathology of small intestine
a - Focal pro-inflammatory cell aggregation with predominant submucosal mononuclear cells
b - Submucosal glandular hyperplasia (black arrow) with atrophic microvilli (blue arrow)
c - Villous necrosis (blue arrow) and epithelial sloughing with crypt hyperplasia (black arrow) d; 100X

a steadily high prevalence in several regions of the globe is linked to poor sanitation, irregular deworming, contaminated feed and water, and resilient parasite eggs. The current research indicated a high rate of single infections, then double and triple infections, in turn. To date, the result is in agreement with Raza *et al.* (2017) in their research in Tabriz, Iran. Previous studies have documented similarly reported that, even in populations where mixed parasitic infections occur in domestic chickens, single type infections are generally more common. The observed patterns of single infection may be attributed to several factors, including inadequate sanitation practices, contaminated feed or water sources. Irregular deworming procedures, as well as the resilience of nematode eggs within the environment, for a long time.

In the present study weather wise, prevalence was higher during the wet season and hot weather, compared to the dry season, which aligns with the study of Anggrahini *et al*. (2025) from Indonesia. Tsegaye *et al*. (2024), and Thomas *et a*l. (2024), who found that the moist and warm conditions enhance parasite egg survival, larval development. Among associated factors, gender-based prevalence rate was more in female chickens as compared to males, which aligns with findings of Saemi Soudkolaei *et al*. (2021) from Iran, who documented the high infection rate in females due to their voracious and unselective feeding nature at egg-laying stage. Further Refisa and Rebuma (2024) and Sarba *et al*. (2020) also reported similar tendencies, associating that females are prone to high parasitic infection due to foraging habits. Subedi *et al.* (2018) also documented the role of gender-related behaviour in influencing parasite burden. Age-based prevalence revealed a higher prevalence rate in adult chickens (> 6 months) as compared to young, which might be due to prolonged exposure to contaminated litter. Which is align with the studies of Shifaw *et al*. (2021), Tay *et al*. (2017), and Raza *et al*. (2014) who found a higher prevalence in adult chickens, possibly due to prolonged exposure to contaminated environment for feeding and drinking. Health-based result shows a higher prevalence rate was noted in weak chickens compared to healthy ones, which aligns with the findings of Wondimu *et al*. (2019), who reported high worm burdens in chickens with weak health, poor immunity, and reduced weight gain. A similar pattern of prevalence was also documented by Jaiswal *et al.* (2020) and Temesgen *et al.* (2024) observed a high prevalence rate in weak chickens with weak immunity and poor hygiene. From a management perspective, nematode infection rates were significantly higher in free-range systems than in semi-free-range systems consistent with the findings of Tsegaye and Miretie (2021) who find out that Scavenging chickens showed a higher prevalence than those provided with supplementary feed, likely due to exposure to contaminated soil containing faeces, and intermediate hosts (Sharma *et al.*, 2017; Ara *et al.*, 2021). The higher risk in free-range systems is mainly due to uncontrolled feeding, dirty living conditions, and close contact with infectious stages.

Table 2. Mebendazole and its derivative in control of nematode infection.

Parasite	Group	Drug	Pre Treatment (Mean ± SD)	Post treatment (Mean ± SD)	Reduction %	*P*-value
Ascaridia galli	X	Control	340 ± 40	370 ± 40	0	0.18
	Y	Derivative	340 ± 50	30.6 ± 50	91	0.0001***
	Z	Mebendazole	340 ± 60	74.8 ± 60	78	0.0001***
Capillaria spp.	X	Control	250 ± 35	265 ± 35	0	0.3
	Y	Derivative	250 ± 45	30 ± 45	88	0.0001***
	Z	Mebendazole	250 ± 55	75 ± 55	70	0.0001***
H. gallinarum	X	Control	280 ± 38	310 ± 38	0	0.13
	Y	Derivative	280 ± 48	28 ± 48	90	0.0001***
	Z	Mebendazole	280 ± 50	72.8 ± 50	74	0.0001***
Trichuris spp.	X	Control	220 ± 32	235 ± 32	0	0.23
	Y	Derivative	220 ± 42	17.6 ± 42	92	0.0001***
	Z	Mebendazole	220 ± 53	55 ± 53	75	0.0001***

SD= Standard deviation ****P* < 0.001 (very highly significant)

Based on Deworming, the prevalence of nematode was higher in non-dewormed chickens compared to in dewormed chickens, which aligns with findings of Velkers *et al*. (2021), Subedi *et al.* (2018), and Berhe *et al.* (2019). He also documented that the infection rate was more common in untreated chickens as compared to treated ones. Regular deworming lowers the internal parasite load, prevents egg shedding, and reduces environmental contamination, thereby breaking the transmission of infection. The multivariate logistic model revealed that gender, age, body condition, weather, and a number of management-related risk factors significantly co-varied with parasitism by gastrointestinal nematodes amongst domestic chickens. Higher risk is most likely connected to increased exposure to filthy environments, lower levels of deworming, and inadequate nutritional supplements in less regulated systems. Histopathological alterations induced by nematode infection revealed the most important pathological changes, including petechial bleeding, mucosal thickening, and intestinal obstruction, which are consistent with findings of Shifaw *et al.* (2021) and Tsegaye and Miretie (2021), who reported that mild parasitic infections may not induce overt pathological changes in the intestinal tissue. Our results suggest that moderate to heavy nematode burdens damage the intestinal wall, reducing feed efficiency, slowing growth, and increasing mortality.

The severity of lesions appears proportionate to the parasite load, as also highlighted by Wuthijaree *et al*. (2019). These pathological changes are mainly caused by mechanical damage from parasite attachment, abrasion of intestinal mucosa, and the release of toxic metabolic by-products, which provoke chronic inflammation and disrupt normal gut function.

The present study showed that Mebendazole and its derivative significantly reduced roundworm burden in chickens over 21 days. The derivative was slightly more effective as compared to mebendazole, as reported that mebendazole disrupts microtubules, blocking glucose uptake and killing parasites (Lassen, 2017). Worm counts dropped by day 14 and further by day 21, similar to Permin (2021), who reported an over 90 % egg reduction rate, revealing 78.6 % efficacy with 50 mg/kg Mebendazole for six days against nematode infection. Despite using a lower dose, our results suggest room for optimized treatment. Fenbendazole, another benzimidazole, achieved over 90 % efficacy with minimal effects on egg production and good pharmacokinetics. In many developing countries, nematode infections in chickens are commonly treated without proper diagnostic evaluation. Resistance has emerged as a consequence of the irregular and intermittent use of anthelmintic drugs (Mitraet *et al*., 2023). Studies conducted by Ritu *et al*. (2024 evidenced that helminths show resistance to anthelminthic drugs, with special reference to *Ascaridia* species often occur alongside other parasites.

Conclusions

The current study investigated that *Ascaridia galli* and *Heterakis gallinarum* were found to be the most prevalent nematode infections in female chickens, sub-adults, week-old body conditions, wet season. Among the free-range, scavenging, non-dewormed animals, there were mostly infected with these nematode infections. Hemorrhagic enteritis, necrotic patches, intestinal inflammation, submucosal oedema, glandular hyperplasia and villous atrophy were noted as pathological changes in the intestinal tissues. Mean number of eggs per gram of faeces *A.galli* was the highest, followed by *H.gallinarum*, *Capillaria* spp., and *Trichuris* spp., respectively. Mebendazole derivative was noted as most effective against nematode infection, significantly reducing the number of eggs per gram of faecal samples (EPG) of roundworm infection.

Conflicting interests

No competing interest is found among the authors related to this study.

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