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Comparative Prevalence of Gastrointestinal Parasites in Free-Range Chickens: A Cross-Sectional Study of Rural (Khotang) Vs. Urban (Dharan) Settings in Eastern Nepal

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Abstract
Gastrointestinal (GI) parasites continue to threaten poultry health and productivity across the developing world, particularly in smallholder and waste-collection-based production systems. Despite the rapid growth of the poultry sector in Nepal, little is known about how parasite burdens differ between rural and urban environments. To help fill this gap, a cross-sectional survey was conducted on 100 free-range chickens, 50 from rural Khotang and 50 from urban Dharan, between 2023 and 2024. Standard faecal flotation and sedimentation techniques were used to detect and identify GI parasites. Infection was widespread in both locations: 78% of rural chickens and 70% of urban chickens tested positive, although this difference was not statistically significant ($p = 0.33$). Helminths predominated in both areas, with *Capillaria* spp. and *Ascaridia galli* being the most commonly detected species. Urban chickens, however, exhibited notably higher rates of *Ascaridia* infections and mixed infestations, suggesting potential biosecurity gaps in peri-urban poultry systems. Protozoan parasites, such as *Eimeria* spp. and *Isospora* spp., were detected less frequently, possibly due to environmental factors or limitations in the diagnostic methods used. No significant difference in parasite distribution at the phylum level was observed between the two sites ($p = 0.74$). Taken together, these findings highlight the need for routine deworming, improved hygiene practices, and targeted farmer education programs. The baseline data generated by this study can contribute to the development of effective poultry health management strategies within the One Health framework.
Keywords: GI parasites, *Capillaria*, *Ascaridia*, Poultry, Nepal, Rural–Urban

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Introduction
Gastrointestinal (GI) parasites are among the most persistent and economically damaging health problems facing poultry farmers worldwide. Their impact is felt through slower growth, poor feed conversion, reduced egg output, and, in severe cases, increased bird mortality (Permin et al., 1999; Ruff, 1999). In

Nepal, where chicken rearing supports both household food security and income, especially in rural communities, parasitic disease remains a serious problem. The reasons are not hard to find: veterinary infrastructure is limited, biosecurity practices are often minimal, and the traditional free-range or semi-scavenging systems that most smallholders rely on create ideal conditions for parasite transmission (Neupane & Kaphle, 2023; Poudel et al., 2023; Rai & Ghimire, 2025).

We know from existing research in Nepal that several helminths and protozoa, including *Ascaridia galli*, *Heterakis gallinarum*, *Raillietina* spp., and *Eimeria* spp., are common in backyard chickens, with regional and seasonal variation in their prevalence (Aljoboury et al., 2024; Das et al., 2022; Sreedevi et al., 2016). What we do not yet know is whether these parasite burdens differ meaningfully between rural and urban settings. This is a more important question than it might first appear: rural and urban poultry systems differ substantially in their housing, feeding, sanitation, and access to veterinary care (Batiso et al., 2019; Fagrach et al., 2023). Understanding how these differences translate into parasite exposure and infection is essential for designing interventions that actually fit local realities. Existing studies from Nepal have documented a range of helminths and protozoa in backyard poultry, *Ascaridia galli*, *Heterakis gallinarum*, *Raillietina* spp., and *Eimeria* spp., among them, with prevalence varying by region and season (Adhikari et al., 2008; Jayswal et al., 2014; D. Shrestha et al., 2020; Subedi et al., 2018). What remains largely unexplored is whether parasite burdens differ between rural and urban settings. The question matters practically: the two systems diverge in housing design, feeding regimens, sanitation standards, and access to veterinary support (Azam et al., 2025; Ferdushy et al., 2016; Mullens & Murillo, 2017), and those contrasts are likely to shape both the intensity and composition of parasite communities. Without empirical data comparing the two, it is difficult to target control efforts appropriately.

Rural chickens in Nepal typically roam freely in backyards and open fields, scavenging for food and regularly coming into contact with soil, water, and invertebrate intermediate hosts—earthworms and insects—that carry infective parasite stages (Jha, 2019; Parajuli et al., 2022; Subedi et al., 2018). Urban chickens, even in semi-intensive settings, often have more structured housing, regulated feeding, and at least occasional veterinary attention (Baldrey et al., 2024; Karabozhilova et al., 2012). These contrasts lead us to expect some difference in parasite prevalence and composition, but empirical data from Nepal comparing the two systems are scarce. In rural Nepal, chickens typically roam freely across backyards and open fields, scavenging and coming into regular contact with soil, water, earthworms, and insects that serve as intermediate hosts for various parasites (Devleesschauwer et al., 2014; Dhakal et al., 2024). In urban settings, even semi-intensive flocks tend to benefit from more structured housing, controlled feeding, and at least intermittent veterinary care (Chaiban et al., n.d.; Kejela et al., 2019). These management differences give good reason to expect that parasite profiles will diverge between the two systems, yet data from Nepal directly comparing them remain scarce.

Eastern Nepal gives us a convenient natural experiment. Dharan is a growing urban center with increasingly semi-commercial poultry production, while Khotang—a remote hilly district to its northwest—retains traditional, subsistence-oriented farming. By surveying chickens in both locations, we can begin to trace how management context shapes GI parasite burdens. Specifically, this study aims to assess and compare the prevalence, species diversity, and infection patterns (single versus mixed infections) of GI parasites in free-range chickens from these two contrasting environments (Acharya et al., 2022). Beyond the local findings, this work contributes to a wider One Health conversation by highlighting the zoonotic risks posed by parasites such as *H. gallinarum*, which can serve as a vector for *Histomonas meleagridis* and other pathogens affecting both poultry and, potentially, human health (Ruff, 1999).This study aims to compare GI parasite prevalence, species diversity, and infection patterns including single versus mixed infections in free-range chickens from Khotang and Dharan. The work also has broader relevance within the One Health framework, since some of the parasites involved carry zoonotic risk: *H. gallinarum*, for instance, acts as a vector for *Histomonas meleagridis* and other pathogens with implications for both poultry health and, potentially, human health (Ruff, 1999).

Materials and Methods

Study area

Dharan is a sub-metropolitan city in Sunsari District, Koshi Province, situated at the foothills of the Mahabharat Range where the hills meet the Terai plains. Lying at roughly 349 m above sea level and covering 192.32 km², it is the largest city by area in Koshi Province and serves as an important hub connecting the hill and Terai regions. The climate is subtropical, with warm temperatures and heavy monsoon rainfall. Poultry-rearing practices here are transitioning toward semi-commercial production.

Khotang District, located northwest of Dharan, is a representative hilly area covering 1,591 km², with elevations ranging from 152 to 3,620 m above sea level. The landscape is mostly forested (56%) and agricultural (42%), and the climate shifts from subtropical at lower elevations to temperate higher up. In recent years, local cooperatives have supported growth in poultry farming as a pathway to self-sufficiency in meat and egg production. This makes Khotang an informative setting in which to examine parasite dynamics in traditional, subsistence-based poultry systems.

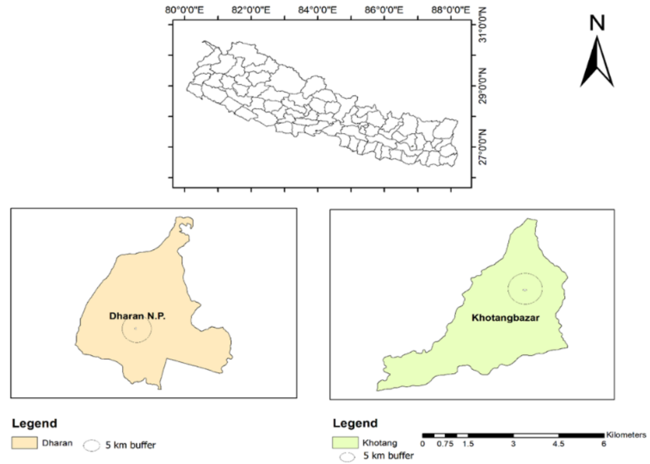

Figure 1. study area

Sample Collection and Preservation

We collected a total of 100 fecal samples and 10 intestinal samples from the two study areas using an open-collection approach (Foreyt, 2001). Faecal samples were transferred with a sterile wooden stick into sterile vials, and 2–3 drops of potassium dichromate were added immediately to preserve helminth cyst morphology and developmental stages. Intestinal (alimentary canal) samples were placed in sealed ziplock in icebags and transported directly to the laboratory.

Laboratory examination

Flotation method

We mixed 1 g of faecal sample with 10 mL of distilled water, homogenized it, and strained the suspension to remove large debris. The strained suspension was centrifuged at 1,000 rpm for 5 minutes, the supernatant discarded, and the pellet resuspended in a supersaturated sodium chloride (NaCl) solution. After a second centrifugation, one drop of methylene blue was added, more NaCl was introduced to create a convex meniscus at the tube rim, and a coverslip was placed on the meniscus for 5–10 minutes to capture helminth eggs and protozoan cysts. The coverslip was then transferred to a glass slide and examined under a light microscope at 10× and 40× magnification (Chatterjee, 1976; Soulsby, 1982; Zajac et al., 2021).

Sedimentation method

For the sedimentation method, 1 g of faecal sample was emulsified in 10 mL of distilled water, strained to remove particulates, and transferred to a centrifuge tube. Following centrifugation at 1,000 rpm for 5 minutes and removal of the supernatant, the sediment was resuspended in normal saline. A small aliquot was mixed with Lugol's iodine solution, covered with a coverslip, and examined microscopically for parasitic structures (Chatterjee, 1976; Soulsby, 1982; Zajac et al., 2021).

Parasite identification

Parasitic eggs, cysts, and larvae were identified by their morphological features shape, size, and color using a trinocular microscope at 10× and 40× magnification. Identifications were cross-checked against published morphological references (Shayan et al., 2016).

Data analysis

All data were analyzed in R (version 4.3.2; R Core Team, 2023). Descriptive statistics summarized overall and species-specific prevalence as well as the distribution of single versus mixed infections. Differences in prevalence, species composition, and infection patterns between the two sites were assessed with chi-square (χ^2) tests, with statistical significance set at $p < 0.05$. Because only 10 intestinal samples were collected, those data were described without inferential statistics.

Results

Overall prevalence of gastrointestinal parasites

We found 74 tested positives out of 100 collected samples from both the study sites, giving an overall prevalence of 74.0% (95% CI: 64.3–81.9%). Khotang had a slightly higher infection rate, with 39 of 50 birds positive (78.0%; 95% CI: 64.8–87.2%), compared to 35 of 50 birds in Dharan (70.0%; 95% CI: 56.2–80.9%) (Table 1).

Although Khotang showed an 8 percentage point higher prevalence, the confidence intervals for both sites overlapped considerably, and the difference was not statistically significant. Chi-square analysis with Yates' correction confirmed no significant difference between sites ($\chi^2 = 0.47$, df = 1, $p = 0.49$), supported by Fisher's exact test ($p = 0.50$). The risk ratio of 1.11 indicates that rural birds were only slightly more likely to be infected than urban birds, suggesting that high parasite burdens can build up in both rural and urban free-range or backyard systems, and that geographic location alone is not a reliable predictor of infection risk.

Species-specific prevalence and parasite composition

We found seven different parasite species across the 100 faecal samples (Table 2). *Capillaria* spp. and *Ascaridia* spp being the most common at both sites. *Strongyloides* sp. was detected only in urban Dharan and showed a significant site difference, while *Heterakis gallinarum* was also found only in Dharan but with borderline significance.

Helminth and protozoan infection patterns

Grouping parasites into helminths (worms) and protozoa showed that worms made up the majority at both sites (Table 3). We did find no significant differences in the helminth-to-protozoan ratio between sites ($p > 0.05$), although protozoan detections were relatively higher in Khotang.

Single and mixed infection patterns

We got the positive cases from single infection to mixed infection among birds in both the sites. The pattern of single versus mixed infections differed considerably (Table 4). In rural Khotang, most positive birds carried only one parasite species: 24 of 39 (61.5%) had single infections, while 15 (38.5%) carried two or more. In urban Dharan, the pattern was reversed: 22 of 35 positive birds (62.9%) had mixed infections, and only 13 (37.1%) carried a single parasite.

Table 1. Overall prevalence of gastrointestinal parasites in free-range chickens from rural Khotang and urban Dharan, eastern Nepal

Study site	No. examined	No. positive	Prevalence (%)	95% CI (%)
Rural (Khotang)	50	39	78.0	64.8–87.2
Urban (Dharan)	50	35	70.0	56.2–80.9
Overall	100	74	74.0	64.3–81.9

Table 2. Species-specific prevalence of gastrointestinal parasites detected in free-range chickens from rural Khotang (n = 50; 39 positive) and urban Dharan (n = 50; 35 positive), eastern Nepal.

Parasite species	Rural n/50	Rural % (all)	Rural % (+ve)	Urban n/50	Urban % (all)	Urban % (+ve)	Fisher p
Capillaria spp.	19/50	38.0	48.7	22/50	44.0	62.9	0.685 NS
Ascaridia galli	13/50	26.0	33.3	14/50	28.0	40.0	1.000 NS
Isospora sp.	14/50	28.0	35.9	7/50	14.0	20.0	0.140 NS
Raillietina tetragona	7/50	14.0	17.9	9/50	18.0	25.7	0.786 NS
Eimeria sp.	4/50	8.0	10.3	6/50	12.0	17.1	0.741 NS
Strongyloides sp.	0/50	0.0	0.0	6/50	12.0	17.1	0.027 **
Heterakis gallinarum	0/50	0.0	0.0	5/50	10.0	14.3	0.056 *
Total detections	57	—	—	69	—	—	All parasites per bird included

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This difference was significant by the uncorrected Pearson's chi-square test (χ^2 = 4.39, df = 1, p = 0.036), but became borderline after Yates' continuity correction (χ^2 = 3.47, p = 0.063), a result supported by Fisher's exact test (p = 0.062).

Intestinal sample findings

Of the 10 intestinal samples examined, we found two samples positive (20.0%) (Table 5). Both positive samples contained *Ascaridia galli*, one from a rural bird in Khotang and one from an urban bird in Dharan.

Table 3. Distribution of helminth and protozoan parasite detections in free-range chickens from rural Khotang and urban Dharan, eastern Nepal.

Parasite group	**Rural detections**	**Urban detections**	**Rural % of total**	**Urban % of total**
Helminths (*Capillaria* spp., *A. galli, R. tetragona*, *Strongyloides* sp., *H. gallinarum*)	39	56	68.4	81.2
Protozoa (*Isospora* sp., *Eimeria* sp.)	18	13	31.6	18.8
Total	57	69	100.0	100.0

Table 4. Distribution of single and mixed gastrointestinal parasite infections among positive free-range chickens from rural Khotang and urban Dharan, eastern Nepal

Site	**Single infection n (%)**	**Mixed infection n (%)**	**Total positive**	**Predominant pattern**
Rural (Khotang)	24 (61.5%)	15 (38.5%)	39	Single
Urban (Dharan)	13 (37.1%)	22 (62.9%)	35	Mixed

Table 5. Results of intestinal examination for gastrointestinal parasites in free-range chickens from rural Khotang and urban Dharan, eastern Nepal.

Sample source	No. examined	No. positive	Positivity rate (%)	Parasite identified
Rural (Khotang)	5	1	20.0	*Ascaridia galli*
Urban (Dharan)	5	1	20.0	*Ascaridia galli*
Overall	10	2	20.0	*Ascaridia galli*

Discussion

Overall GI parasite prevalence was high (74.0%), confirming that gastrointestinal parasitism remains widespread in Nepalese free-range and backyard poultry irrespective of rural (Khotang) or peri-urban (Dharan) setting. This prevalence aligns with previous national surveys and supports the view that GI parasites represent an endemic, persistent constraint on smallholder poultry health that requires sustained control rather than one-off treatments (Devleesschauwer et al., 2014; D. Shrestha et al., 2020).

Contrary to our initial expectation, overall prevalence (Khotang 78.0% vs Dharan 70.0%), the helminth-to-protozoan ratio, and phylum-level parasite distributions did not differ significantly between sites. This suggests that management practices and environmental exposure rather than geographic classification as “rural” or “urban” determine parasite risk, consistent with (Berihun et al. 2026). Semi-commercial peri-urban systems in Dharan likely still permit substantial contact with contaminated soil, refuse, and intermediate hosts, sustaining transmission.

At species level, *Capillaria* spp. and *Ascaridia galli* predominated at both sites, reflecting their direct, soil borne transmission and concordance with earlier Nepalese studies (Devleesschauwer et al., 2014; A. Shrestha et al., 2007). *Strongyloides* sp. and *Heterakis gallinarum* were detected only in Dharan, with *Strongyloides* reaching statistical significance. The presence of *H. gallinarum*, although of low prevalence, is notable because of its role as a vector for *Histomonas meleagridis* and its One Health implications (Ruff, 1999). These urban-exclusive detections may indicate peri-urban biosecurity gaps (e.g., market introductions, mixed species keeping) that merit targeted monitoring.

Mixed infections were more common in Dharan (62.9% of positive birds) than Khotang (61.5% single infections), a difference that remained suggestive across tests despite attenuation after Yates' correction. Higher co-infection rates may reflect greater exposure heterogeneity in peri-urban flocks (higher bird density, shared scavenging), and have practical relevance because polyparasitism can increase host burden and complicate treatment efficacy (Permin et al., 1999).

Intestinal examinations (n = 10) recovered *A. galli* in one bird per site, corroborating faecal findings and supporting the field diagnostic approach (Shayan et al., 2016). However, the small number of necropsies limits inference on infection intensity.

Study limitations include the cross-sectional design that omits seasonal variation, diagnostic methods (flotation/sedimentation) that may underdetect protozoa and light infections, and modest site sample sizes (n = 50) that reduce power to detect differences in rarer species. Future research should employ repeated seasonal sampling, larger cohorts, and more sensitive diagnostics (e.g., PCR) to refine species-level identification and temporal dynamics.

Conclusion

This study compared gastrointestinal (GI) parasite prevalence, species diversity, and infection patterns in free-range chickens from rural Khotang and urban Dharan. Overall prevalence was similar: 75% of birds at both sites harboured ≥1 GI parasite, with helminths predominating and *Capillaria* spp. and *Ascaridia galli* the most frequent species. Dharan exhibited a higher frequency of mixed infections and was the sole site where *Strongyloides* sp. and *Heterakis gallinarum* were recorded; the latter is notable as a vector for *Histomonas meleagridis* and for One Health concerns. Findings indicate that peri-urban systems do not necessarily reduce parasite risk and can exhibit distinct infection profiles. We recommend unified control measures across settings: regular deworming, improved litter and housing hygiene, and farmer biosecurity education (quarantine new birds, limit shared scavenging) with species-specific adjustments guided by surveillance. The prevalence and species composition data reported here provide a baseline for intervention design and monitoring in Nepal's evolving poultry sector.

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Conflicts of Interest

The authors declare no conflicts of interest related to this study.

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