PREVALENCE OF GASTROINTESTINAL PARASITES IN FREE RANGING SAKINI CHICKEN (*Gallus gallus domesticus* Linnaeus, 1758), KIRTIPUR, KATHMANDU, NEPAL

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A dissertation submitted

in partial fulfilment of the requirements for the degree of Master of Science in Zoology with special paper Parasitology.

Central Department of Zoology
Institute of Science and Technology
Tribhuvan University
Kirtipur, Kathmandu

July, 2025

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Declaration

I hereby declare that the work presented in this dissertation "**Prevalence of gastrointestinal parasites in free ranging Sakini Chicken (*Gallus gallus domesticus* Linnaeus, 1758), Kirtipur, Kathmandu, Nepal**" has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author(s) or institution(s).

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Recommendation

This is to recommend that the dissertation entitled "**Prevalence of gastrointestinal parasites in free ranging Sakini Chicken (*Gallus gallus domesticus* Linnaeus, 1758), Kirtipur, Kathmandu, Nepal**" has been carried out by Susma Rana for the partial fulfilment of Master's Degree of Science in Zoology with special paper Parasitology. This is her original work and has been carried out under my supervision. To the best of my knowledge, this dissertation work has not been submitted for any other degree in any institutions.

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Letter of approval

On the recommendation of supervisor "**Janak Raj Subedi**" this dissertation submitted by Susma Rana entitled "**Prevalence of gastrointestinal parasites in free ranging Sakini Chicken (*Gallus gallus domesticus* Linnaeus, 1758), Kirtipur, Kathmandu, Nepal**" is approved for the examination in partial fulfilment of the requirements for Master's Degree of Science in Zoology with special paper Parasitology.

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Certificate of acceptance

This dissertation work submitted by Susma Rana, entitled "**Prevalence of gastrointestinal parasites in free ranging Sakini Chicken (*Gallus gallus domesticus* Linnaeus, 1758), Kirtipur, Kathmandu, Nepal**" has been accepted as a partial fulfilment for the requirements of Master's Degree of Science in Zoology with special paper Parasitology.

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Susma Rana

Abstract

This study investigated the prevalence of gastrointestinal parasites in free-ranging Sakini Chicken (*Gallus gallus domesticus* Linnaeus, 1758), in Kirtipur, Kathmandu, Nepal, focusing on the types of parasitic infections and the effectiveness of anthelminthic treatment. A total of 171 fecal samples of poultry were collected from a small backyard farm in eight months from September 2024 to April 2025, developed in Kirtipur. These fecal samples analyzed using direct smear, floatation, and sedimentation methods. The results revealed one protozoan and four nematode parasitic infections. The prevalence of parasites showed *Eimeria* sp. (28.65%), *Capillaria* sp. (8.77%), *Heterakis* sp. (5.65%), Strongyle and *Asaridia* sp. (3.51%). Parasitic prevalence was found the highest in November and December (66.67% each). The gastrointestinal parasites have strong relation ($\chi2$= 1.00 to 0.23; $P>0.05$), in November to March except in April ($\chi2$= 0.01; $P<0.05$). There was a significantly association between helminth parasite and anthelminthic treatment of parasites ($\chi2$= 0.02; $P<0.05$). The drugs were very effective to *Ascaridia* and *Strongyle* and relatively less effective in *Heterakis* and *Capillaria* species. This research will contribute to Nepal's "Envisioning Nepal 2030" initiative by addressing key Sustainable Development Goals such as poverty reduction, food security, health, and economic growth in the poultry sector. The findings conclude the need for improved integrated parasite management practices, including better litter management, targeted treatments, and further research on drug resistance and protozoan control.

शोध सारांश

यस अध्ययनले काठमाडौंको किर्तिपुरमा स्वतन्त्र रूपमा पालिने साकिनी कुखुरा (*Gallus gallus domesticus* Linnaeus, 1758) मा हुने सुलीमा परजीवीको प्रसार, तिनको प्रकार र कृमिनाशक औषधिको प्रभावकारिता माथि केन्द्रित गरेको छ। अध्ययनमा किर्तिपुरको एक सानो फार्मबाट मध्य-भाद्र २०८१ देखि मध्य-वैसाख २०८२ सम्म ८ महिनामा जम्मा १७१ वटा कुखुराको मलका नमूनाहरू संकलन गरिएको थियो। यी नमूनाहरू प्रत्यक्ष स्मियर, फ्लोटेसन र सेडिमेन्टेसन विधिबाट परीक्षण गरिएको थियो। नतिजामा एक प्रोटोजोआ र चार नेमाटोड परजीवी संक्रमण पाइयो**:** *Eimeria* sp. **(**२८.६५%)**,** *Capillaria* sp. (८.७७%)**,** *Heterakis* sp. (५.६५%)**,** *Strongyle* र *Ascaridia* sp. (३.५१%)। कार्तिक देखि फाल्गुनमा परजीवीको प्रसार सबैभन्दा बढी (६६.६७%) रहेको देखियो। परजीवीहरू कार्तिक देखि फाल्गुनसम्म (χ^2= १.०० देखि ०.२३; P>०.०५) सम्बन्ध देखिएको थियो, तर चैत्र- वैसाखमा यसको सम्बन्ध कमजोर (χ^2= ०.०१; P<०.०५) रहेको थियो। कृमिनाशक औषधिको प्रभावकारिता *Ascaridia* र *Strongyle* मा धेरै प्रभावकारी देखिएको भने *Heterakis* र *Capillaria* मा केही कम प्रभावकारी रहेको पाइयो। यस अनुसन्धानले नेपालको " Envisioning Nepal 2030" अभियानमा महत्त्वपूर्ण योगदान पुर्याउनेछ। यसले दिगो विकास लक्ष्यका प्रमुख पक्षहरू जस्तै: गरिबी न्यूनीकरण, खाद्य सुरक्षा, स्वास्थ्य र कुखुरा पालन क्षेत्रमा आर्थिक वृद्धिलाई सम्बोधन गर्दछ। अनुसन्धानका नतिजाले एकीकृत परजीवी व्यवस्थापन प्रणाली सुधारको आवश्यकता रेखाङ्कित गर्दछ, जसमा: सुली व्यवस्थापन, लक्षित उपचार, औषधि प्रतिरोधकता र प्रोटोजोआ नियन्त्रण सम्बन्धी थप अनुसन्धान गर्नुपर्ने देखिन्छ, जसले कुखुरा पालन क्षेत्रलाई अधिक उत्पादक, स्वस्थ र आर्थिक रूपमा लाभकारी बनाउन मद्दत गर्नेछ।

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List of abbreviations

Abbreviated form	Details of abbreviations
AEZs	Agro-Ecological Zones
CDZ	Central Department of Zoology
cm	Centimeter
Etc	Et cetera
FAO	Animal Health Manual
FPPS	Family Poultry Production System
gm	gram
Max	Maximum
Min	Minium
NADIL	National Avian Disease Investigation Laboratory
NPC	National Planning Commission
rpm	Revolution per minute
sp.	Species
temp	Temperature
VTH	Veterinary Teaching Hospital

1. Introduction

1.1 Background

Agriculture is the backbone of the developing country like Nepal where more than 55% people are depending on it. Among them contribution of agriculture sector in the national GDP is 21% and about 4% of only from the poultry subsection of the livestock contribution. Indigenous poultry covers about 55% of the total poultry population (MoALD, 2023). Therefore, poultry helps in the economic development of the country and livelihood of Rural families in Nepal including family income, employment, food security and daily protein intake, drinking water and the sanitation and the overall development (Dhakal, 2019).

Indegenous chicken breeds of Nepal are Sakini, Khuile and Dumsi (Pwankh Ulte) (Jegede et al., 2016). These chickens are found different agro- ecological zones (AEZs) of Nepal (Sapkota, 2017) and raised as family poultry production system (FPPS) (Gorkhali et al., 2021). The Sakini chicken breed demonstrates promising genetic potential for egg production, with an annual yield of 140 eggs per hen in hilly regions. Its eggs show favorable physical parameters, averaging 47.45g total weight with well-balanced components: 13.6g yolk (28.7% of egg weight) and 29.28g albumen (61.7%). These characteristics - consistent laying capacity combined with optimal egg composition - make Sakini an ideal candidate for selective breeding programs aimed at enhancing productivity in Nepal's poultry sector. The breed's adaptation to hilly conditions further increases its value for small-scale poultry farmers seeking reliable egg production in challenging terrains. These quantifiable traits provide a strong foundation for genetic improvement initiatives targeting both egg quantity and quality. (Gorkhali et al., 2021). Indigenous chickens as backyard farming with productive and reproductive performance was analyzed by Paneru, 2016. His findings about average age first lay egg is 161.62 days, clutch size 2.89 and hatchability 74%. The nacked neck breed shows better performance in hilly region and Sakini in the Terai. The poultry business is mostly affected by social factors such as age of farmers, family size and No. of laying birds. The net return is high in large farm where higher mass production of eggs and lower feed conversion rate signify the profit margin (Osti et al., 2016).

There are different types of rearing systems that depends on purpose, quantity and location. The local chickens in Nepal usually raised as free range that feed in natural habitat by scavenging habits, mostly in the dung sites, fields and open spaces such as grass land or cultivation area where the rarely used ready-made feeds and lack of proper medication. Free-range chickens face considerable predation risks, as numerous wild and domestic animals threaten their survival. Natural predators such as crows, hawks and foxes along with household pets including dogs and cats, frequently attack scavenging poultry. These predation incidents, coupled with potential theft, create major obstacles for free-range chicken farming. Small-scale producers particularly suffer heavy economic losses due to these security challenges in open-range production systems. Seasonal outbreaks create the loss of economy and spreads different types of diseases to human too. As the chickens are known as host for different parasites that may be protozoan to helminths. The gastrointestinal parasites are most harmful to both farmer and consumer. It creates most of the loses in the poultry farms due to the loss of body weight, decreasing eggs number, very less fertility and ultimately the loss of money to the farmers (Jha, 2019). Similarly, for the consumers that transmits different types of disease due to consumption of raw meat or not properly cooked meat and meat products.

1.2 Statement of problem

There is plenty proper research on the topic of gastrointestinal parasites infection in free range chicken and backyard poultry farm. But lacks in Nepalese scenario due to which the farmers are not aware about this problem. As a result, there is huge economic loss in the poultry sector.

1.3 Objectives

1.3.1 General objective

This study aims at investigation of prevalence of gastro-intestinal parasites on the free ranging Sakini chicken (*Gallus gallus domesticus* Linnaeus, 1758).

1.3.2 Specific objectives

The specific objectives of the study are listed below:

- To ascertain the frequency of intestinal parasites in chickens raised on the free ran ging
- To study the effectiveness of anti-helminth drugs against parasites in free-range chickens.

1.4 Research question

- What are the gastrointestinal parasites in local chickens?
- Which species of gastrointestinal parasites are more prevalent to backyard and free-range chickens?
- Does the prevalence rate of gastrointestinal parasite in free range chickens affect after anti-gastrointestinal medicine provided?

1.5 Significance of the study

There is no proper record keeping in small chicken farm, this is due to lack of knowledge, careless or both. As a consequence, diseases and parasites that greatly affect the livelihood and sanitation of the farmer. This study is carried out to document such type of study like presence of gastrointestinal parasites, and effectiveness of anti-gastrointestinal medicine in the free-range chicken.

This study adds value in "Envisioning Nepal 2030" a National Planning Commission (NPC) initiative to chart a long-term development strategy for Nepal, aiming to graduate from the Least Developed Country (LDC) status, become a middle-income country by 2030, and achieve the Sustainable Development Goals (SDGs). The main focus on agriculture in the poultry sector to fulfil the goals such as:

a. No poverty

b. Zero Hunger

c. Good health and well being

1.6 Limitations of the study

Poultry science encompasses diverse aspects including health, nutrition, and management. This study's findings are localized, being limited to a single small-scale farm in Kirtipur over eight months. While providing valuable farm-specific insights into parasite prevalence and treatment efficacy, the results may not represent broader regional patterns due to the constrained scope. Such focused research serves as an important preliminary investigation, though wider studies across multiple farms and seasons would be needed for more generalizable conclusions about Nepal's poultry sector. The study nevertheless offers practical insights for improving small-scale poultry health management.

2. Literature review

2.1 Literature review related to free range chickens

A study on village chicken production practices in South Africa by (Nyoni, 2012) appeared that bigger portion (93.8%; n = 76) of their family units additionally given a couple of outline of secure for their chickens. In show disdain toward of the reality that, most respondents (93.8%; n = 76) certified the utilize of elective cures to control parasites and treat illnesses; most chicken gatekeepers (81.5%; n = 66) experienced chicken incidents due to predation and prosperity related issues.

The study on GI parasites of chickens in India by (Singh et al., 2021) appeared that coccidiosis was the overwhelming disease among all GIT parasites based on fecal and intestinal tract substance investigation. The hazard components related with the predominance of GIT parasitic diseases were topographical area, profound litter framework, broilers, age, crossbred breeds, and storm season.

The study by (Fanatico et al., 2013) on Free-choice feeding of free-range chickens showed that a free-choice nourishing may be a strategy that provides feathered creatures partitioned feedstuffs, such as grains, protein concentrates, and normal vitamin and mineral sources, from which they can self-select a slim down suited to their changing needs. Free-choice nourishing may demonstrate valuable in generation frameworks with open air get to since supplement needs alter broadly due to temperature variances and feathered creature movement.

The study of (Acharya & Kaphle, 2015) on sustainable poultry development in Nepal highlights three critical focus areas: research on locally adaptable production technologies, development of suitable poultry breeds, and improved management practices. Their work establishes a new framework for poultry farming programs tailored to Nepal's diverse agro-ecological zones.

2.2 Literature review related to parasites

According to season, the prevalence rate was lowest (14%) in the fall and greatest (33 %) in the spring and summer. The age-wise prevalence was the highest (48%) in the 31-45 dayss age group and the least (6%) in 0-15 days age group of layers. Coccidiosis was shown to be more common in farms with mud/mudbrick floors than i n those with concrete floors (Adhikari et al., 2009).

Study by (Permin et al., 1999) on Danish poultry production systems revealed significant variation in gastrointestinal helminth prevalence across different rearing methods. Their research demonstrated that free-range and backyard flocks faced the highest infection risks due to direct soil contact and intermediate host exposure, while deep litter systems showed intermediate prevalence from accumulated organic matter. Conventional cage systems exhibited the lowest parasitism, highlighting how management practices directly influence parasite transmission dynamics. These findings established foundational knowledge about production system-specific parasitism that remains relevant for modern poultry health strategies.

From April 2011 to March 2012, researchers examined 10 chickens monthly (totaling 120 hosts) to assess: (1) prevalence rates, (2) mean infection intensity, and (3) species diversity of cestodes. The year-long sampling across seasonal variations provides comprehensive data on tapeworm burdens in this temperate, high-altitude poultry production system, where free-ranging management increases parasite exposure risks. Findings offer valuable baseline parasitological information for this understudied Himalayan region. (Dar & Tanveer, 2013). A parasitological survey of 120 free-range chickens (70 hens, 50 cocks) across Kashmir revealed cestode infections in 103 birds (85.83%), indicating widespread tapeworm prevalence in the region's backyard poultry. (Dube et al., 2010) investigated parasitic infections in free-range chickens (*Gallus domesticus*) across Zimbabwe's Matebeleland regions, employing salt flotation and microscopic fecal analysis to identify intestinal parasites. Their research documented nine ectoparasite species infesting poultry, including feather lice *(Menopon gallinae, Menacanthus stramineus*), mites (*Dermanyssus gallinae*, *Cnemidocoptes mutans*), fleas (*Echidnophaga gallinacean*), and ticks (*Argas persicus*). These parasitic burdens correlated with significant production challenges, particularly reduced hatchability rates and impaired growth performance in affected flocks. The study's findings highlight how combined endo- and ectoparasite infestations can substantially compromise poultry productivity in smallholder farming systems. The study on histopathological of tapeworm *Raillietina tetragona* (Molin, 1858) from the gastro-intestine of indigenous chicken farming in Kirtipur was done by (Jha, 2019). He found that the helminth infections are endemic in the study area, as 19. Irungu et al., 2004 investigated helminth parasites in indigenous Kenyan poultry, identifying significant gastrointestinal burdens in free-ranging systems. The study reported high prevalence of nematodes (*Ascaridia* *galli*, *Heterakis gallinarum*) and cestodes (*Raillietina* spp.), with infections linked to scavenging behavior and poor parasite control. Notably, *A. galli* dominated, reflecting its direct life cycle and resilience in tropical climates. The findings underscore how traditional management practices—such as unrestricted foraging and infrequent deworming—exacerbate parasitism, leading to reduced productivity (weight loss, low egg yields). This work provides critical baseline data on helminth diversity and risk factors in Kenya's smallholder poultry systems, highlighting the need for affordable, culturally adapted control measures. (Rabbi et al., 1970) compared gastrointestinal helminth infections across poultry production systems, finding highest burdens in free-range chickens (primarily Ascaridia galli and Heterakis spp.), intermediate levels in deep-litter systems (Raillietina spp.), and lowest in cages. The study linked these differences to environmental exposure, housing conditions, and age (peak susceptibility at 6-12 weeks), providing evidence for system-specific parasite control strategies in poultry operations.

The information gathered from this study will help to improve the overall productivity and profitability of the poultry industry in Kathmandu. Point predominance think about of gastro-intestinal parasites in town chickens of Centane locale, South Africa was done by (Mwale & Masika, 2011). Seventy families were arbitrarily chosen for compound testing of new fecal tests of chickens. Adjusted quantitative McMaster method was utilized for examination of nematode and cestode eggs, and coccidial oocysts. Subjective sedimentation procedure was utilized to identify trematode eggs. The hens have various intestinal parasites in about 99 percent of cases. *Hetarakis gallinarum* was the foremost predominant (25.72% Qolora by-sea and 27.14% Nontshinga) beside coccidia (41.43% Qolora by-sea and 25.71% Nontshinga town). Trematodes and cestodes were the slightest predominant. Butt et al., 2014 investigated cestode parasites in local chickens (*Gallus domesticus*) from Hyderabad, Pakistan, revealing significant infection rates tied to free-range management. The study identified *Raillietina tetragona* and *R. echinobothrida* as predominant species, with higher prevalence during humid months (July–September) due to increased intermediate host (ants, beetles) activity. Risk factors included scavenging behavior, poor litter management, and lack of deworming protocols. These findings highlight how traditional poultry systems in Sindh foster cestode transmission, necessitating integrated control measures combining husbandry improvements and targeted anthelmintic use.

(M. N. Alam et al., 2014) documented gastrointestinal helminth infections in indigenous chickens across Barisal District, Bangladesh, revealing high parasitism in scavenging systems. The study identified *Ascaridia galli* (41.6%) as the most prevalent nematode, followed by *Heterakis gallinarum* (18.3%) and *Capillaria* spp. (9.7%), with cestodes (*Raillietina* spp.) detected in 12.4% of birds. Prevalence correlated with poor sanitation, monsoon-driven humidity, and lack of deworming practices factors exacerbating parasite transmission in smallholder farms. These findings underscore the need for integrated parasite management in Bangladesh's backyard poultry systems, where helminths reduce productivity. (Kumar et al., 2015) found coccidiosis (*Eimeria* spp.: 52.6%) to be the most prevalent parasitic infection in Upper Gangetic Plains poultry, with *E. tenella* and *E. maxima* predominating. Concurrent helminth infections (*A. galli*: 34.2%; *Raillietina*: 18.9%) were common in free-range systems, exacerbated by monsoon conditions (>70% humidity) and poor management. The study reported 20-25% chick mortality, underscoring the need for integrated control combining housing improvements and targeted antiparasitics in smallholder systems. (Jatau et al., 2016) discovered three genetically distinct *Eimeria* OTUs in 62% of Nigerian chicken flocks, which often co-occur with *E. tenella* and *E. maxima*. These variants evade standard PCR diagnostics due to sequence divergence, risking false negatives. The findings emphasize the need for localized genomic data to improve coccidiosis detection and control in Nigeria. (Fatoba & Adeleke, 2018) reviewed coccidiosis management, advocating integrated approaches combining molecular diagnostics (qPCR, LAMP), vaccination, and drug rotation to address strain-specificity and resistance. The study highlighted the need for regionally-adapted strategies incorporating live vaccines, synthetic anticoccidials, and improved farm hygiene, particularly in resource-limited areas requiring affordable diagnostics and sustainable control solutions.

2.3 Literature review related to anthelmintic

There are many literatures available on commercial anthelmintic drugs as well organic treatments in the under developed countries.

The combined use of ivermectin and *Nigella sativa* (black seed) extract showed enhanced anthelmintic efficacy against helminths in Aseel chickens compared to individual treatments. This synergistic effect suggests that bioactive compounds in black seed (e.g., thymoquinone) may potentiate ivermectin's action, offering a sustainable, resistance-mitigating approach for small-scale poultry farmers in Pakistan. The findings support integrating herbal and conventional deworming strategies in native poultry production (Pafčo et al., 2018). A field study in Iran evaluated the efficacy of two common anthelmintics – fenbendazole (a benzimidazole) and levamisole (an imidazothiazole) – in naturally infected local chickens. Both drugs demonstrated significant effectiveness against gastrointestinal nematodes under free-range conditions, though fenbendazole showed marginally superior efficacy (85-92% parasite reduction vs. levamisole's 80-87%). This comparative performance data provides practical guidance for Iranian poultry farmers: fenbendazole may be preferable for intensive parasite control, while levamisole remains a viable alternative, particularly given potential differences in regional parasite resistance patterns. The findings are especially relevant for small-scale producers managing indigenous chicken breeds with high parasite exposure from scavenging behavior. By quantifying treatment outcomes in local conditions, the study enables evidence-based deworming decisions that balance efficacy, cost, and resistance management for sustainable backyard poultry production (Soudkolaei et al., 2021). The real-world impact of deworming interventions (e.g., albendazole, ivermectin) on smallholder chicken production in Odisha, India studied by (Bessell et al., 2019). It assesses key outcomes such as poultry mortality reduction, weight gain improvements, and egg production increases post-treatment. By comparing treated and untreated flocks, it quantifies the potential income benefits for resource-poor farmers, while highlighting the need for tailored parasite control strategies in India’s small-scale poultry systems.

The study of (Alam et al., 2014) compares herbal remedies (like neem, garlic, and black seed) with modern dewormers (like albendazole) for treating intestinal worms in poultry, finding that while synthetic drugs work faster (90%+ effectiveness), herbal options offer safer, sustainable alternatives (70-85% effective), suggesting farmers could benefit from combining both approaches for optimal parasite control. Chege et al., 2017 evaluated and compared the effectiveness of three common dewormers - piperazine citrate, levamisole hydrochloride, and albendazole - in treating naturally occurring gastrointestinal worms in chickens. The results showed that albendazole was the most effective, followed by levamisole, while piperazine citrate had the lowest efficacy against common poultry worms like *Ascaridia galli* and *Heterakis gallinarum*. The findings help poultry farmers and veterinarians choose the best deworming strategy based on infection type and drug availability. Recent research highlights two potent natural dewormers for poultry: papaya seeds (*Carica papaya*) and squash seeds (*Cucurbita moschata*). Papaya seeds outperform due to their dual-action papain enzymes and carpaine alkaloids, while squash seeds act via cucurbitacin-induced paralysis. Both offer affordable, sustainable alternatives to synthetic dewormers, with added benefits—papaya seeds also enhance growth, gut health, and antimicrobial activity (Sugiharto, 2020). Heat treatment optimizes safety and efficacy. The Nicaraguan study (Skallerup et al., 2005) revealed that gastrointestinal helminth infections significantly impair growth performance in free-range chickens, reducing weight gain by 15-20% in infected birds. However, researchers demonstrated that dietary protein supplementation (20% soybean meal) effectively mitigated these losses, with supplemented chickens achieving 92-95% of the growth rates of parasite-free counterparts. This nutrition-parasite interaction offers smallholders a dual-pathway approach: while deworming remains important, strategic feeding with affordable protein sources (like locally available soybean) can maintain productivity even in parasite-endemic conditions. The findings are particularly valuable for resource-limited tropical farming systems where complete parasite eradication is challenging, providing farmers with a sustainable buffer against production losses through improved nutrition management rather than relying solely on anthelmintics.

3. Materials and methods

3.1 Study area

The study was located in Pragatinagar, Kirtipur near to the capital city Kathmandu, Bagmati Province of Nepal. The study area lies in the South of Tribhuvan University and about 4 km away from the Central Department of Zoology. The coordinates of the site are 27˚ 40' 3.939" and 85° 15' 58.50" respectively.

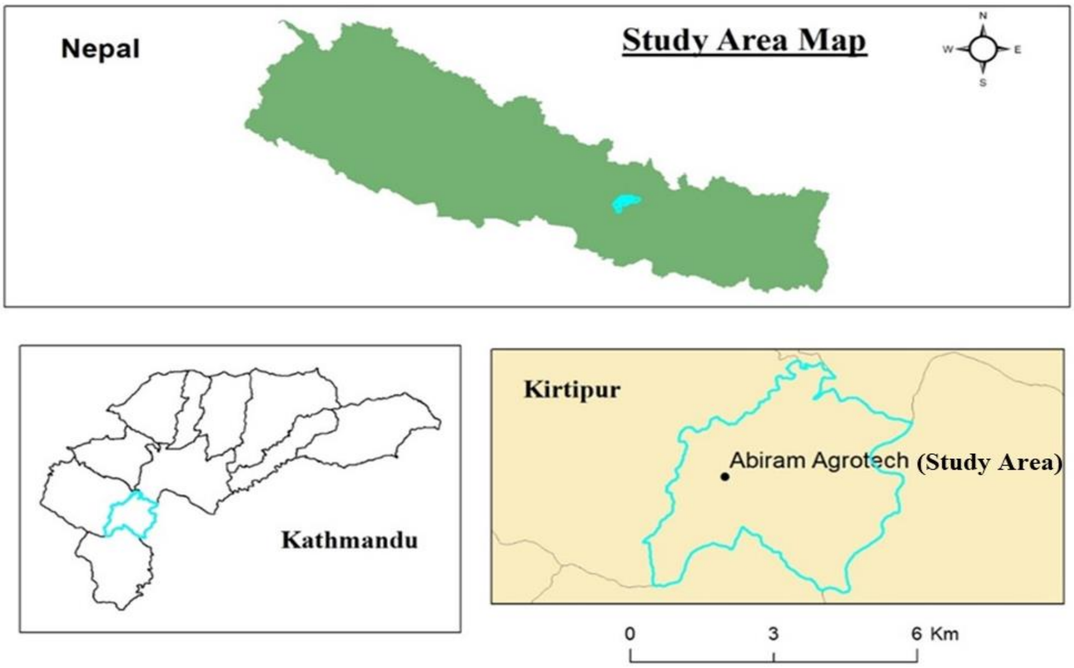


Figure 1. Location map of the study area

3.1.1 Weather

Daytime temperatures fluctuated seasonally, ranging from 28°C (September) to 18°C (December) and reaching 30°C by April. Nighttime temperatures showed greater variation, dropping to 2°C in January after starting at 17°C in September, then rising to 11°C in April. The study period recorded significant rainfall in September (40.8 cm) and October (11.6 cm), driving a gradual decline in humidity from 92% to 87%. Concurrently, as temperatures rose from 2°C to 11°C, relative humidity dropped sharply from 73% to 54%, demonstrating an inverse relationship between temperature and moisture retention. These fluctuations likely influenced parasite survival and transmission dynamics higher September humidity favored protozoan/oocyst persistence, while October's drier, warmer conditions may have reduced environmental larval viability.

3.2. Material required

3.2.1 Field Materials

These field materials are used for field work such as: Sterile vials, Medical Gloves, Polythene Bags, Spatula, Camera, Note book, Marker and ball pen.

3.2.2 Laboratory Materials

Laboratory materials are Gloves and mask, slips, Spatula, Petri disc, Beaker, Tea strainer, Measuring cylinder, Cotton bud, Toothpicks, Centrifuge tube, Test-tube stand, Centrifuge machine, Dropper, Refrigerator, Glass slides and Electric microscope.

3.2.3 Chemicals

i. 2.5% Potassium dichromate ($K_2Cr_2O_7$) solution

ii. Distilled water

3.3. Methods

3.3.1 Development of a Farm

The coop was made for shelter in night and the protection from the predates. It was made using locally available raw materials like bamboo, nails, metal wires & wire mesh, plywood, plastics and mud of the field. It has two stories and deep litter system. The filed mud or clay used as insulating material mixed with water and rice husks on the floor as tradition in the backyard farm in the remote villages of Nepal. There was bamboo perches fixed just one foot from the floor for fowls to rest and sleep. The rice hulls are used for bedding material in the litter and all the droppings collected. The three side walls of coops were made by plywood. These protect from direct air from the environment and keep the inner environment warm. The remaining one side is protected from wire mesh and door for food supplement, cleaning and entrance in the regular manner. The plastic feeder and drinkers were installed for the feeding, water and medicine supply.

3.3.2 Size of Grazing Area

The grazing area for the fowls is 300 square meter (9.3 Aana in Nepali) including 40 square meter of working space for Abiram Agro-Tech. The land was previously used for rice farming and other crops by the owner. There was use of Chicken Litters, cow manures and chemical fertilizers for vegetables growing after the production of rice. It is the lowland and groundwater level is just a foot from the surface and reaches up to three feet in the dry season. The rainwater from the highlands of the road flow through this land in the rainy day. The poultry enclosure is secured by masonry walls on three sides—north, west, and south while the eastern side is enclosed with metal sheets. The fencing varies in height from 2 to 5 feet, providing partial protection. However, a notable vulnerability exists in the southwest corner, where a 2.25-square-foot drainage hole allows rainwater runoff. Unfortunately, this opening also serves as an entry point for predators such as stray dogs, cats, and potentially other small carnivores, compromising the safety of the flock.

3.3.3 Brooding

The Sakini breed chicks were brooded 6 weeks in a wooden brooder of size 4*4 square foot. The floor of the brooder was filled with old, clean clothes for a week that kept the chick's feet stable the movement. Later, the cloth was replaced with rice husks. The temperature was maintained in the coops using 200-watt infrared bulb controlled by W3001 digital thermostat. The temperature was fixed 34-35˚C in the first week and decreased 2°C per week and 25°C in the last week. The many chicks around 50% dead within brooding period, by the symptoms of pasty vents. All these dead chicks were disposed burying 40 cm depth and covered with soil to prevent digging by other animals like street dogs.

3.3.4 Feed

The checks were fed commercial starter feed (locally known as B0 and B1) up to 6 weeks (in the brooding phase) later by mixing mash feed locally made in the mill and in the six months it was mixed with commercial layer feed L3 (7:3 ratio).

3.3.5 Grazing

After 6 weeks, chickens transition to controlled scavenging—released twice daily (4-6 hours) to forage in crop fields. Their diet includes insects, earthworms, grasses, and kitchen waste, reducing feed costs while providing natural nutrition. This system balances welfare (behavioral enrichment) and productivity, though requires predator monitoring and may need supplemental feeding during lean seasons.

3.3.6 Deworming

There are variety of commercial anthelmintic drugs for poultry available globally in both syrup, powder and tablets. Based on the mode of action, these are classified into 5 subclasses from class I to class V (Permin and Hansen, 1998). These are:

Class I anthelmintic e.g. Albendazole, Fenbendazole

Class II anthelmintic e.g. Levamisol

Class III anthelmintic e.g. Piperazine

Class IV anthelmintic e.g. Salicylanilids

Class V anthelmintic e.g. Dichlorvos

Among these, only class I-III are available in the local veterinary shops of Kirtipur for local poultry as rarely farmers use these drugs. The medicines were bought from the veterinary shop of nearby market. These drugs were added in water in in the 3rd week of the month before cleaning the coops in the morning of about 10 am. The quantity & type of medicine is mentioned in the table (Appendix-13).

The Albendazole syrup for the deworming of helminths was used on 21st January, 2025. The syrup was well shake for uniform distribution of medicine content and mixed of 60 ml of syrup in 5L fresh water. The medicine was supplied to all the thirsty chickens. Similarly, another deworming drug Levamisol of 5 gm powder was made solution by mixing it with 5L water. This solution was also supplied to thirty chickens through the drinker on 21st February, 2025. The Levamisol tablet 300mg was pulverized and made solution in water and supplied to the chickens by the same procedure on 21st March, 2025.

3.3.7 **Litter Management**

The deep litter was collected and cleaned thoroughly using tools such as shovels and dust pan every month of third week. These litter was collected in the sacs; most of sold and remaining were used in the field as manure for the vegetables growing. Again, it was filled with new rice husk in the coops.

3.4 Study Design

This investigation sought to analyze the seriousness of intestinal worm infections in farmed chickens. The Gantt chart for the study is in Appendix-12. The study consists of the following steps:

a) Farm development
b) Buying specific chicks and brooding them up to 6 weeks.
c) Grazing the chickens in the compounded land in both morning and evening i. e. twice a day.
d) Collection of fresh fecal samples in the sterile glass vials.
e) Preserving fecal samples in a solution containing 2.5% potassium dichromate.
f) Examining feces samples through the use of direct smear, sedimentation, and flotation procedures.
g) Identification of parasite eggs and cysts and Data analysis through MS-Excel

3.4.1 Sampling collection method

The study collected fresh fecal samples from Sakini chickens during peak defecation hours (8-10 AM) over six days. Researchers promptly gathered 20-25g droppings immediately after excretion to prevent ground contamination. Using disposable gloves, they ensured hygienic sample collection while maintaining parasite integrity. This morning-specific timing capitalized on natural parasite egg shedding patterns, optimizing detection accuracy for laboratory analysis. The standardized protocol (consistent timing, quantity, and handling) minimized pre-analytical variables that could affect parasite identification.

3.4.2 Preservation of fecal samples

The researchers carefully collected chicken droppings and stored them in clean, labeled bottles. To keep the parasites in the samples from changing or growing, they added a special preservative a 2.5% potassium dichromate solution (made by mixing 2.5 grams of the powder in 1 liter of distilled water). This liquid helped maintain the original shape of tiny protozoan parasites and stopped worm eggs/larvae from developing further. The team made sure to collect both semi-solid and solid stool samples properly. Finally, the preserved samples were delivered to the Central Department of Zoology for detailed analysis under microscopes.

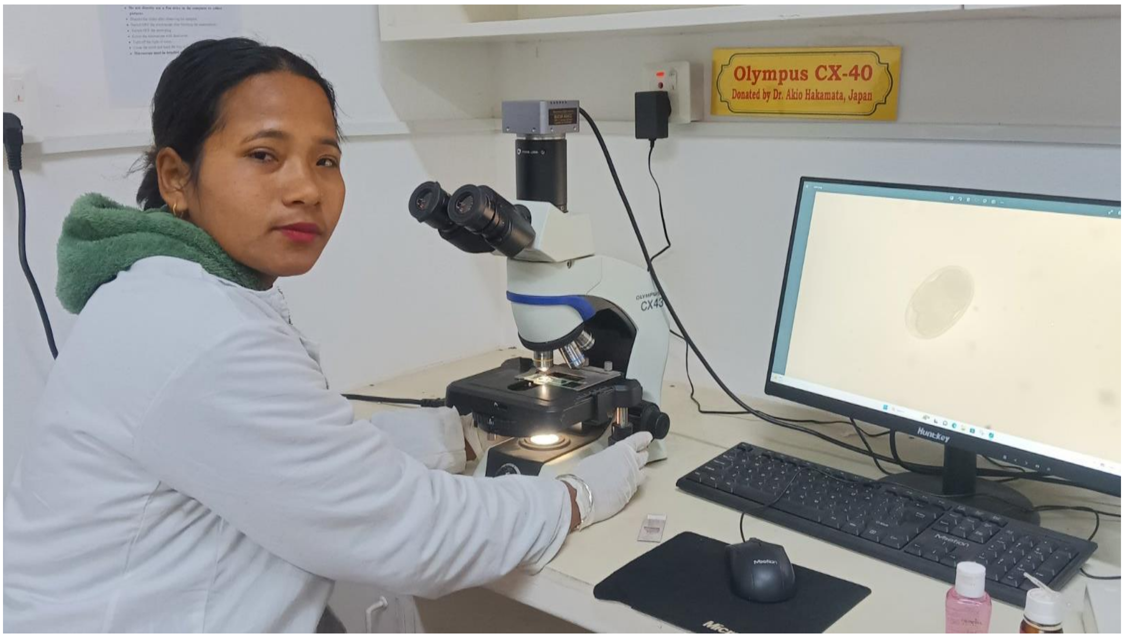
3.4.3 Sample size

This study examined gastrointestinal parasites in 50 Sakini chickens from a Kirtipur farm, collecting 171 fecal samples (60% of the flock monthly) from November 2024 to March 2025. The high sampling proportion ensured accurate parasite detection across seasons, with laboratory analysis using direct smear, flotation, and sedimentation methods. This intensive sampling strategy (around 30 chickens monthly) provided reliable data on infection patterns in Nepal's free-range poultry systems, crucial for developing targeted parasite control measures in small-scale farms. The approach balanced scientific rigor with practical field constraints while capturing potential seasonal variations in parasite prevalence. The sample collection with label is shown Photograph 1.

Photograph 1. Faecal Sample collection at the site

3.4.4 Laboratory Examination and Identification

The fecal samples were processed using concentration techniques (flotation and sedimentation) to enhance parasite detection. Through microscopic examination (shown in Photograph 2), parasite stages (ova, oocysts, cysts, larvae) were identified based on morphological characteristics (size, shape, shell features). These methods allowed both qualitative identification and quantitative estimation of gastrointestinal parasites present in the Sakini chickens. (Zajac et al. 2012).



Photograph 2. Microscopic examination of the collected samples in the CDZ laboratory

3.4.1 Concentration Methods

Eggs/cysts/larvae of parasites can be easily found in smears only in heavy infections. However, very often they are difficult to detect in direct smears of mounts due to their low numbers in faces. Therefore, floatation and sedimentation techniques were used for concentration (Zajac et al., 2012).

3.4.2 Floatation technique

This method exploits density differences, where lighter parasite eggs/cysts float atop dense solutions while debris sediments. Processed fecal samples were first homogenized with water and filtered through a tea strainer to remove large particulates. The filtrate was transferred to 15 mL centrifuge tubes and spun at 1000 rpm for 5 minutes. After discarding the supernatant, the pellet was re-suspended in a saturated NaCl solution (specific gravity ~1.20) and re-centrifuged. To enhance egg recovery, additional NaCl solution was added to form a convex meniscus, and a drop of methylene blue improved contrast. A coverslip placed on the meniscus for 5 minutes trapped floating parasites, which were then transferred to a slide for microscopic examination (10X and 40X magnification). Digital photo microscopy aided in morphological identification of eggs/cysts based on size, shape, and shell features.

3.4.3 Sedimentation techniques

This procedure was based on the rule that parasitic eggs having tall thickness than the suspended medium settle and concentrate at the foot of the medium. The supernatant arrangement of floatation was evacuated and the dregs at the foot was taken out with 8 long pipette, iodine damp mount was arranged for each test by blending 1-2 drops of the silt with Lugol's iodine arrangement on a glass slide. It watched beneath inspected beneath 10X and 40 X magnifying instrument amplifications.

3.4.4 Identifications of eggs and larvae of parasites

Slides arranged utilizing different strategies was inspected beneath a magnifying lens at 10X and 40X separately. Estimation of eggs/oocysts/cysts was measured through arrange and visual micrometer. Distinguishing proof was done utilizing book (Zajac et al., 2012), other distributed and published articles and web sources based on morphological characters (shape and size).

4. Results

During the study period, a total of 171 fecal samples of local chickens were taken from a farm in Kirtipur municipality of Kathmandu district and examined by using saline wet mount method, floatation method and sedimentation method.

4.1. General Prevalence

Out of 171 fecal samples examined, 86 fecal samples were positive for one or more specific GI parasites, showing 50.29% prevalence of parasitic infection whereas 49.71% fecal samples were negative (figure 2).

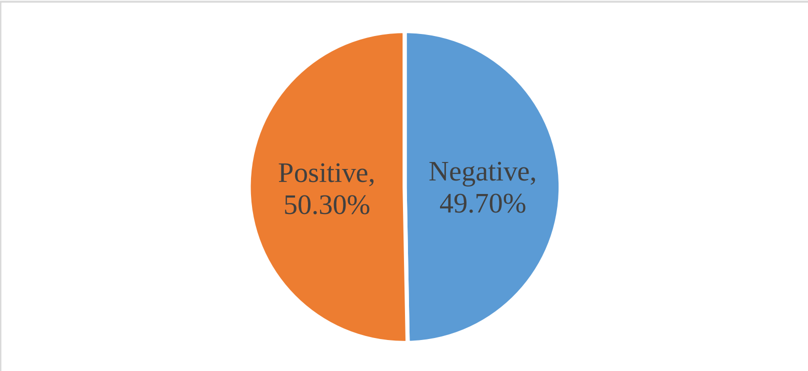


Figure 2 General prevalence of parasites

4.2 Prevalence of specific gastrointestinal parasites

Out of 171 total samples, a total of four nematodes (21.64 %) and one protozoan (28.65 %) parasitic eggs were isolated and identified. The prevalence rate of *Eimeria* sp. was 49 (28.65%) followed by four helminths: *Capillaria* sp. 15 (8.77%), *Heterakis* sp. 9 (5.26%), *Strongyle* sp. 7 (4.09 %) *and Ascaridia* sp. 6 (3.51%) (figure 3).

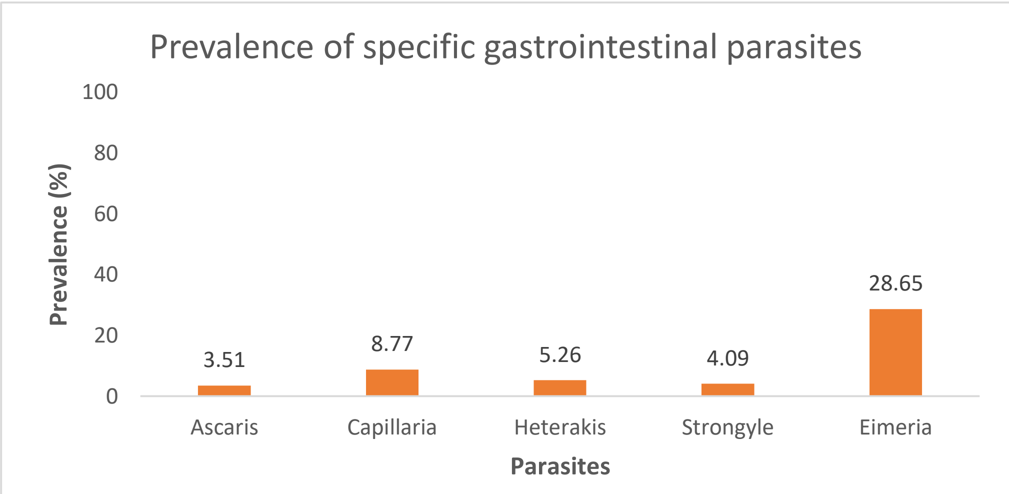


Figure 3 Prevalence of specific parasites

4.3 Monthly prevalence of gastrointestinal parasites

The month wise prevalence of gastrointestinal parasites is 66.67% in November & December, 55.17% in January, 30.77% in February, 43.33% in March and 34.62% in April respectively (figure 4).

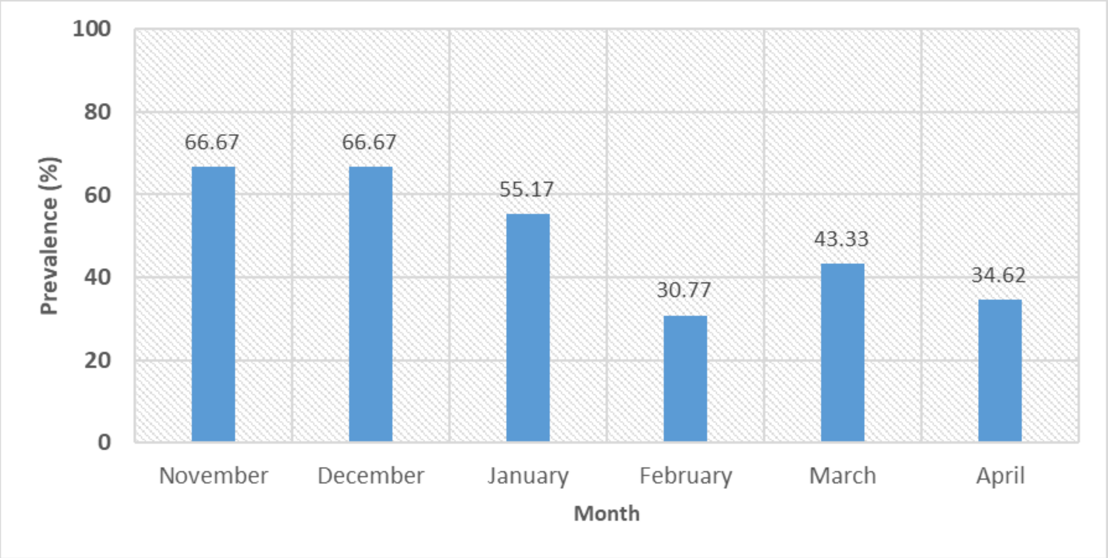


Figure 4 Month wise prevalence of gastrointestinal parasites

4.4 Class wise prevalence of gastrointestinal parasites

The study revealed distinct seasonal patterns in gastrointestinal parasite prevalence among Sakini chickens (Figure 5). Protozoan infections peaked during December-January (up to 10%), likely due to favorable humid conditions for oocyst survival, before declining to 5% in February's drier weather. Helminth infections showed parallel trends, with nematodes reaching 7.02% prevalence in December but nearly disappearing (0.58%) by April, suggesting temperature-dependent larval mortality. These fluctuations demonstrate how environmental factors - particularly humidity and temperature - directly influence parasite transmission dynamics in free-range systems. The findings highlight December as a critical intervention period, when both protozoan and helminth infections concurrently peak, necessitating targeted parasite control measures before monsoon conditions ideal for transmission emerge. There was statistical association of the prevalence of intestinal parasites of protozoa and nematodes in November ($\chi2$= 1.00; P>0.05), December ($\chi2$= 0.66; P>0.05), January ($\chi2$=0.23; P>0.05), February ($\chi2$= 0.44; P>0.05), and March ($\chi2$= 0.75; P>0.05), & no association of protozoa and helminths in April ($\chi2$= 0.01; P<0.05) (figure 5).

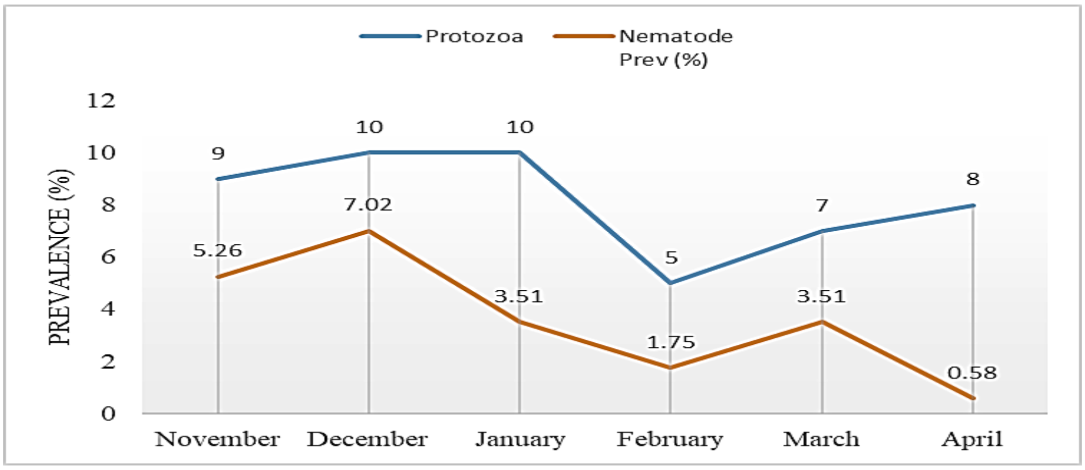


Figure 5 Class wise prevalence of gastrointestinal parasites.

4.5 Effectiveness of deworming drug

The effectiveness of deworming drug was conducted in the local chickens. The drug was given in the last three months: January, February and March prior to a week ago of fecal samples was taken. There was significantly relation to that of deworm drug and the parasites ($\chi2$= 0.02; P<0.05) but these drugs were lest effective on parasites *Capillaria* and *Strongyle* species. At the same time, these drugs had no effect on the protozoan parasites (figure 6).

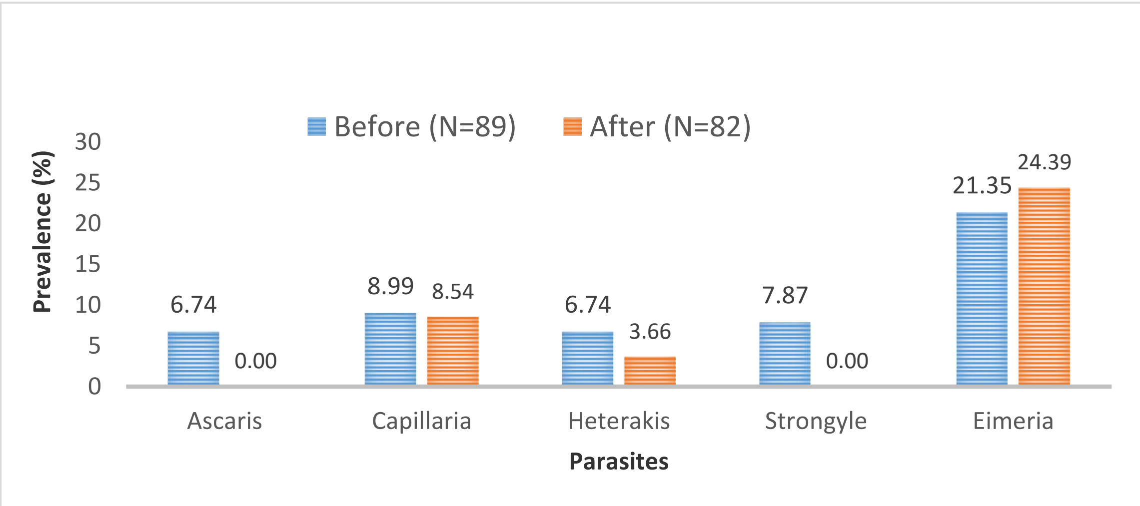


Figure 6 Effectiveness of deworming drugs

The study found no significant association between protozoan and helminth infections in Sakini chickens (χ^2=0.03, p<0.05), indicating independent transmission pathways. This necessitates separate control strategies for each parasite type in free-range systems, where both can coexist without interaction. (figure 6).

5. Discussion

50.29% of chickens (86 out of 171 fecal samples) were infected with gastrointestinal (GI) parasites among them Protozoan (*Eimeria* sp.) was the most prevalent (28.65%), followed by nematodes (*Capillaria* sp., *Heterakis* sp., *Strongyle* sp., *Ascaridia* sp.). The highest infection rates in November and December (66.67%) and declining to 34.62% by April. The infections of protozoa were peaked in December (5.85%), while helminth infections were highest in December (7.02%) and March (3.51%). There was no significant reduction in parasites found after deworming ($\chi^2 = 0.02$; $P < 0.05$) in different last three months. The drug had no effect on protozoans (*Eimeria* sp.) & limited efficacy against helminths (*Capillaria* and *Strongyle* spp. persisted). The statistical analysis showed no significant link between protozoan and helminth infections in most months ($P > 0.05$), except April ($\chi^2 = 0.01$; $P < 0.05$).

There is various study by (Gautam et al., 2017, Bhusal et al., 2023, Panth, 2019, Jha, 2019, different locations of Nepal such as Chitwan, Lalitpur, Kirtipur and Kathmandu. Most of the poultry cases are preferred in the governmental diagnostic center where mostly smaller farms were preferred (Bhusal et al. 2018). The most prevalent and endemic gastrointestinal infections in the poultry sector is colibacillosis found by different researchers such as Gautam et. al 2017, Bhusal et. al 2018, Panth et al. 2019 and (Gompo et al., 2019). The protozoan infection by *Escherichia coli* found all the year due to poor farm hygiene in the poultry house and ultimately the poor poultry farm management. This research had result of the colibacillosis only 1% and differ from other authors such as (Gautam et al., 2017) (39% winter and 14% autum), 27.4% by Bhusal et al. 2018 and 26% by Gompo et al. 2019.

There are plenty of research on the gastrointestinal parasitic infections of poultry sector worldwide and some of them are mentioned in this section. There are 87% overall infections and 66% mixed infection in the country chicken of Andhra Pradesh India by the author (Mathews et al., 2024). The found 83% cestodes, 70.33% nematodes and 0.67% trematodes. There were 100% infection in the July month. These findings are quite differing in this research where there is no Trematodes that need water medium for complete life cycle.

A parasitological survey of indigenous (deshi) chickens in Bangalore, India, revealed significant gastrointestinal parasite burdens, with tapeworms (cestodes) showing the highest infection rate at 52.2%, followed by roundworms (nematodes) at 34.3%. Nearly one-fifth (18.3%) of examined birds harbored concurrent infections with both parasite types. These findings demonstrate: (1) the substantial parasite pressure in Bangalore's backyard poultry systems, (2) the particular success of cestode transmission in this environment likely due to abundant intermediate hosts, and (3) the common occurrence of polyparasitism that may compound negative health effects on chicken productivity. Puttalakshmamma et al., 2008 that results quite differ to the recent study. Katoch et al., 2012 supports to this study where they found the most prevalent gastrointestinal parasites as *Ascaridia* in the backyard chickens of subtropical and humid zone of Jammu, India. Similarly, (Kumar et al., 2015) discovered that 3.45% of the chickens in India's upper Gangetic plains have Heterakis Spp. The research in the local chickens of Hyderabad, Sindh, Pakistan by Butt et al., 2014 that the cestodes parasites of overall infection 94.5% due to the substandard poultry farming that differ to the recent study as the chickens are caged and routinely freed for feeding in the morning and evening and supports the authors Singh et al. 2021 findings in central plain zone of Punjab, India where overall gastrointestinal parasites in poultry of 38.36%, most dominance of coccidia in the deep litter system 54.4%, than cage system 37.5%. The high infection in the crossbred (45.55%) than those of deshi birds (20%) and to the present study had less gender ratio i.e. male is less than that of females. Backyard chickens in and around Shimoga, India 73.2% infection by gastrointestinal parasites where 51.36% cestodes, 28.96% nematodes and 19.67% mixed (Cestodes and Nematodes) by the authors (Javaregowda et al., 2016). Research findings indicate that Thai indigenous chickens raised in backyard farming systems show infection with at least one gastrointestinal helminth parasite species. Wuthijaree et al., 2017.

According to a 2012 study by Bachaya et al., the Ascaria rate at the government poultr y farm in Dina, Punjab, Pakistan, is 24.5%. Prevalence of endoparasites in the domestic chickens across elevation gradient in Java, Indonesia has high prevalence in lowlands (70%) and low in highlands (48%) and diversity of endoparasites is low. A 2015 Europe-wide study Thapa et al., found high helminth prevalence in organic laying hens: *Ascaridia galli* (69.5%), *Heterakis* spp. (29%), and *Raillietina* spp. (13.6%). Outdoor access and organic restrictions on deworming drove these infections, compromising productivity and health. The findings underscore the need for non-chemical parasite control in organic poultry systems.

A parasitological study conducted in Bangladesh's Barisal district revealed significant gastrointestinal helminth infections in indigenous chickens, identifying six parasitic species: five nematodes and one cestode. The research found *Ascaridia galli* to be the dominant parasite, affecting 41.56% of birds, likely due to its direct life cycle and efficient transmission in free-range systems. In contrast, *Dispharynx spiralis* showed minimal prevalence (1.56%), possibly reflecting its complex life cycle requiring intermediate hosts (e.g., arthropods) that may be less available in this ecosystem. Alam et al., 2014. Backyard chickens of Khuzdar, Baluchistan, Pakistan has 53.50% gastrointestinal parasitic infections, where female chickens (72%) are more infections than that of males (35%). The older chickens are more susceptible to the infections 56% than younger 52% (Bajoi et al., 2024). This is due to the older needs more energy based on the body size and physiological activities. Similarly, the authors in the research area of Narsingdi district of Bangladesh, the backyard chickens have high cestode infections 86-92% than nematode (70-86%) (Ferdushy et al., 2013) and no protozoan parasites infection mention. That the researcher could focus only in the helminths parasites. Globally, poultry are affected by approximately 30 helminth species, with *Ascaridia galli* (35%), *Heterakis gallinarum* (28.5%), *Capillaria* spp. (5.9%), and *Raillietina* spp. (1.9%) being most prevalent, as reported by Shifaw et al., 2021. While many studies rely on postmortem examinations for parasite detection, this study's lower prevalence rates may reflect its exclusive use of morning fecal sampling during scavenging. This methodological difference could underestimate true infections because: (1) parasite egg shedding follows diurnal rhythms, and (2) scavenging may dilute fecal samples with environmental debris. The approach, though non-lethal and field-friendly, potentially misses parasites localized in intestinal tissues or those with intermittent egg release patterns. The study highlights endemic parasitic infections in free-range chickens**,** with seasonal peaks and ineffective deworming practices**.** Addressing these issues requires integrated parasite management (hygiene, diagnostics, and drug rotation). Further research on anthelmintic resistance and protozoan control is recommended**.**

6. Conclusions and recommendations

6.1. Conclusions

This study found that Sakini chickens of a backyard farm in, Kirtipur, Kathmandu, Nepal, were infected with various gastrointestinal parasites, including protozoa and worms. The most common gastrointestinal parasites infecting the poultry population were *Ascaridia* sp., *Eimeria* sp., *Capillaria* sp., and *Heterakis* sp. Notably, no fluke (trematode) and tapeworms (cestode) infections were detected. The overall gastrointestinal parasite infection rate was high. The protozoan infections were low to moderate while nematodes were low to very low. There were noticeable monthly variations in parasitic infections. The study also revealed no significant association between helminth and protozoan parasites in the examined chickens, indicating these two parasite groups infect their hosts independently of each other. This independence suggests distinct transmission dynamics and ecological requirements for each parasite type. Helminths (such *as Capillaria* and *Heterakis* species) and protozoans (particularly Eimeria) appear to follow separate infection patterns, potentially due to differences in their life cycles, environmental survival strategies, or host immune responses. The only exception occurred in April, where a weak but statistically significant association (χ^2 = 0.01; $P < 0.05$) was detected, possibly reflecting seasonal variations in host exposure or parasite biology. These findings have important implications for parasite control, as they demonstrate that interventions targeting helminths may not necessarily affect protozoan infections, and vice versa. Consequently, integrated parasite management strategies should address both parasite groups separately to ensure comprehensive protection for poultry health.

6.2. Recommendations

The study is limited to a single farm only. Therefore, quantity of samples should be more for the statistical analysis of data. It is recommending to increase the no. of samples and either raising more chickens or by collection samples from different farms focusing on gender wise, age wise, elevation wise, breed wise and molecular study for the species of parasites.

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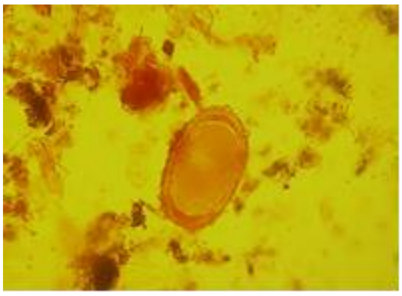
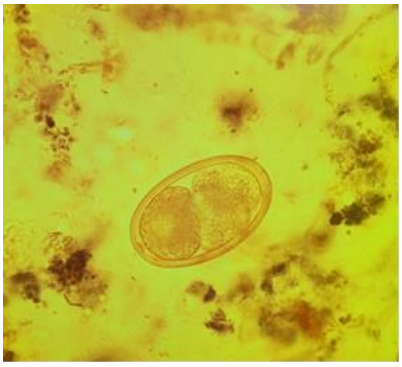
Appendices

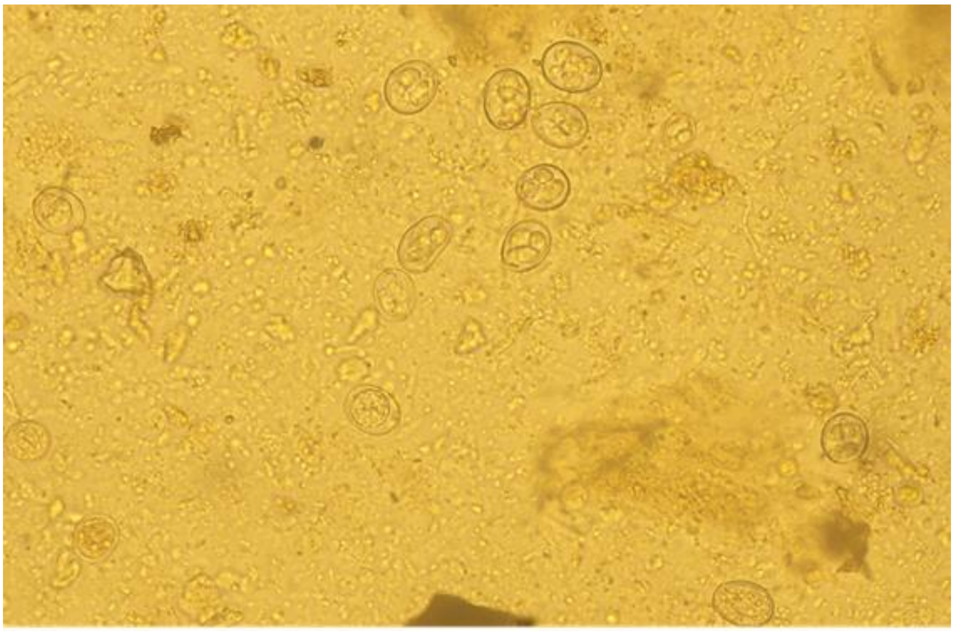
Appendix 1. Some photographs of Gastrointestinal parasites under the microscopic Examination



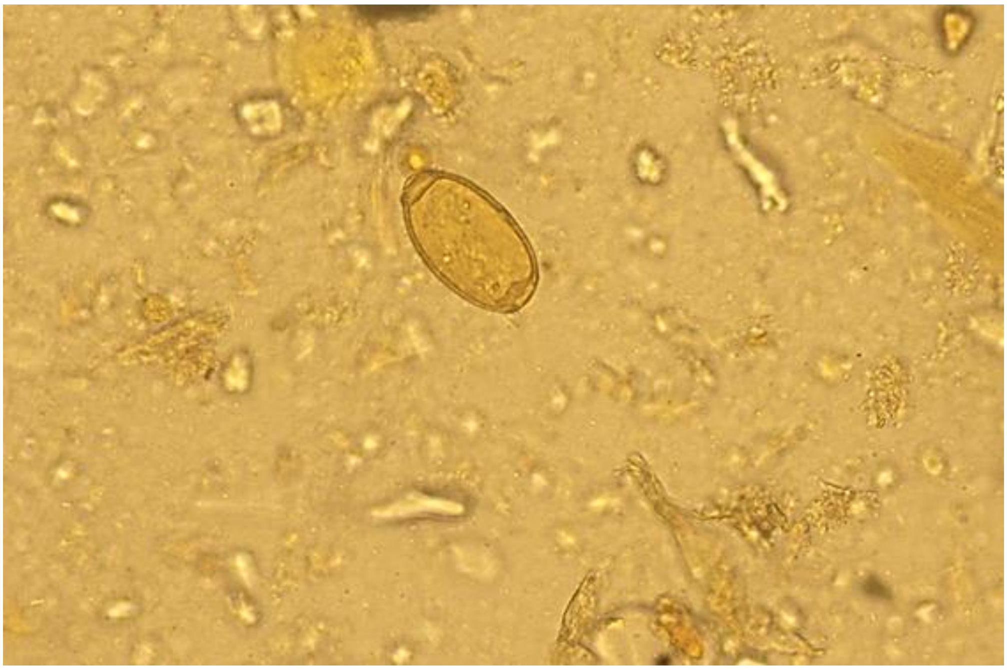

Photograph 3. Gastrointestinal parasites *Ascaris* (82 µm* 52µm; Left-single cell stage & Right- Four cell stage)

Photograph 4. Gastrointestinal parasites *Heterakis* Sp (75µm *48µm; Left-single cell stage & Right- Four cell stage)



Photograph 5. Gastrointestinal parasites *Eimeria* Spp (17µm *13µm)



Photograph 6. Gastrointestinal parasites *Capillaria* sp. (45µm * 25µm)

Figure 7 Gastrointestinal Parasites Strongyle sp. under 40X (40µm* 30µm)

Appendix-2. Sample collection in the farm

Photograph 6. Fecal Sample with container

Photograph 7 Samples at site after collection

Appendix-4 Feeding and Scavenging in the backyard space (Hen was eating earthworm)

Photograph 8 Female chicken eating earthworm during grazing

Photograph 9 Male and female chickens in the crop field

Appendix-5 Litter in the chicken coop

Photograph 10 Litter in the chicken coops

Appendix-6 Deworming drugs

Photograph 11 Deworming drugs applied for chickens

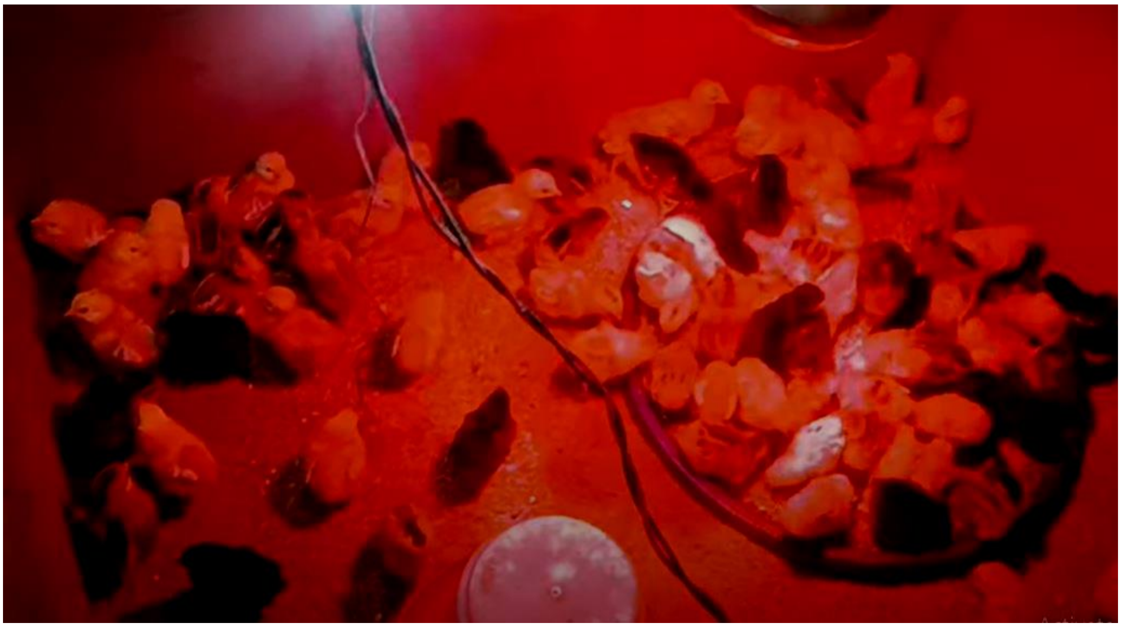
Appendix-7 Fecal matter of mouse in the chicken feeder

Photograph 12 Mouse litter in the chicken feeder

Appendix-8 Crow in the scavenging field

Photograph 13 Crow dwelling for food in the grazing land

Appendix-9 Brooding chicks



Photograph 14 Electric brooder for baby chicks

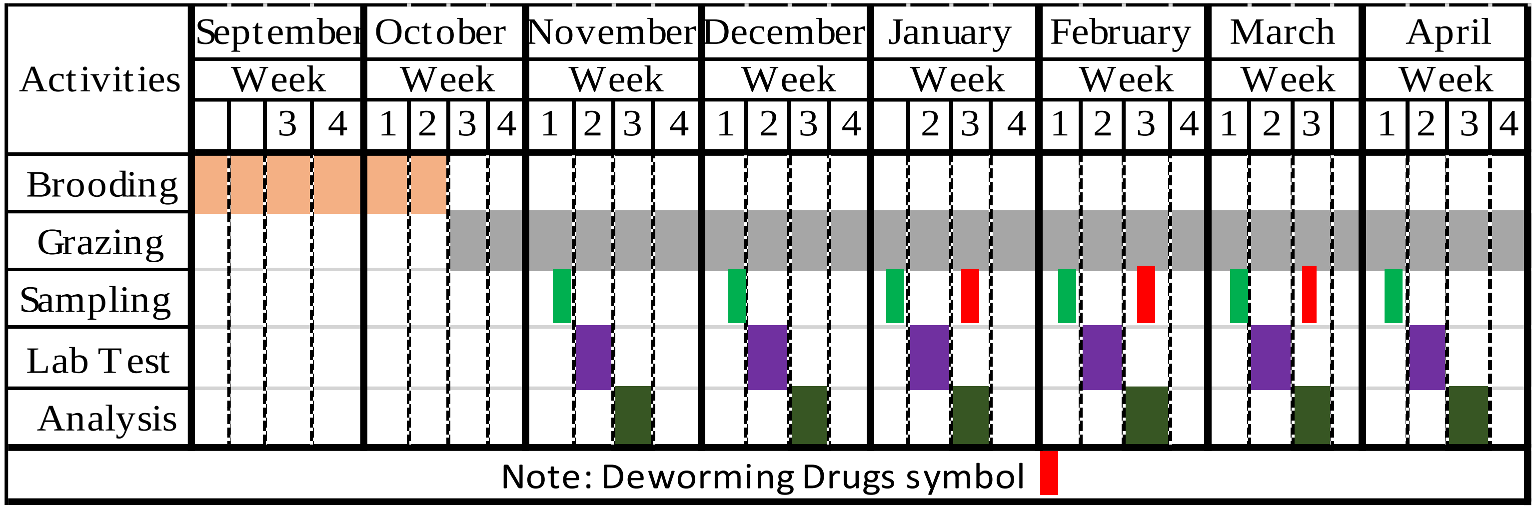
Appendix-10 Infected Chick and disposing the dead

Photograph 15 Infected dead chicks

Appendix-11 Scavenging habitat

Photograph 16 Scavenging in the grassy area of the land

Appendix -12 Gantt chart for the study design



Appendix -13 Deworming drugs given to the Sakini Chickens

Date	Age of chickens (months)	Quantity	Type of medicine
Jan 21 (Magh 8)	5	1 ml	Albendazole Syrup
Feb 21 (Falgun 9)	6	5 gm	Levamisol Powder
Mar 21 (Chaitra 8)	7	10 mg	Levamisol Tablet