Prevalence of Gastrointestinal Parasites of Ring Necked Pheasant (*Phasianus colchicus*) in Dang Valley, Nepal

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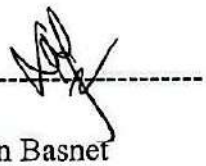
Declaration

I hereby declare that the work presented in this dissertation "Prevalence of gastrointestinal parasites of ring necked pheasants in Dang valley, 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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On the recommendation of supervisor "Dr. Kishor Pandey" and co-supervisor "Mr. Janak Raj Subedi" this dissertation submitted by Robin Basnet entitled "Prevalence of gastrointestinal parasites of ring necked pheasants in Dang valley, 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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Recommendation

This is to recommend that the dissertation entitled "Prevalence of gastrointestinal parasites of ring necked pheasants in Dang valley, Nepal" has been carried out by Robin Basnet for the partial fulfilment of Master's Degree of Science in Zoology with special paper Parasitology. This is his/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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Certificate of acceptance

This dissertation work submitted by Robin Basnet entitled "Prevalence of gastrointestinal parasites of ring necked pheasants in Dang valley, 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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Abstract

The ring-necked pheasant (*Phasianus colchicus*) is a game bird originally from South Asia and is commonly found in countries such as Pakistan, India, Thailand, and Nepal. Nepal is home to various wild pheasant species while the practice of raising pheasants for human consumption is a relatively new agricultural practice in the country. The purpose of this study was to determine the common gastrointestinal parasites in the pheasants of three municipality of Dang Valley, Nepal. A total of 150 fresh fecal samples 75 from male pheasants and 75 from females were collected and preserved in a 2.5% potassium dichromate solution. These samples were subjected to direct smear, flotation and sedimentation techniques for microscopic examination at 100× and 400× magnification. Data were analyzed using Venn diagrams, tables and bar charts and statistical analysis was carried out using R Studio. Of the 150 samples analyzed, 66 tested positive for parasitic infections, indicating an overall prevalence of 44%. Five different parasitic genera were identified in the ring-necked pheasants. Among nematodes, *Heterakis* spp. (12.66%), *Capillaria* spp. (8%), *Ascaridia* spp. (6%), and *Strongyle* spp. (5.33%) were detected. For protozoa only *Eimeria* spp. (12%) was identified. The prevalence of parasitic infection was higher in male pheasants (49.3%) compared to females (38.66%), although the difference was not statistically significant ($p = 0.26$). Additionally, the prevalence was highest at lower altitudes (48%) and lowest at higher altitudes (36%), with no significant statistical difference between the two altitudes ($p = 0.222$). Statistically, the difference in prevalence of intestinal parasitic infection among study area was found to be insignificant ($p=0.103$). Overall, the findings suggest that ring-necked pheasants in the Dang Valley are susceptible to gastrointestinal parasites. So to minimize the GI parasite in ring necked pheasant routine fecal examination and deworming should be carried out.

शोध सारांश

रिङ-नेक्ड कालिज (*Phasianus colchicus*) दक्षिण एसियाको स्थायी खेल चराहरू मध्ये एक हो । यो प्रायः पाकिस्तान, भारत, थाईल्यान्ड र नेपालमा पाइन्छ । नेपालमा धेरै प्रकारका जंगली कालिज प्रजातिहरू पाइन्छन्, तर मानव उपभोगको लागि बन्दी अवस्थामा कालिज पालन गर्नु हालसालैको कृषि अभ्यास हो । यस अध्ययनको उद्देश्य नेपालको दाङ उपत्यकामा रिङ-नेक्ड कालिजका पेटमा रहेका परजीवीहरूको व्यापकताको अनुसन्धान गर्नु थियो । कुल १५० ताजा दिशाको नमूना संकलन गरियो, जसमा ७५ वटा भाले र ७५ वटा पोथी समावेश थिए । यी नमूनाहरुलाई २.५% पोटासियम डाइक्रोमेटमा संरक्षित गरियो । नमूनाहरुलाई माइक्रोस्कोपद्वारा प्रत्यक्ष स्मियर विधि, फ्लोटेशन र सेडिमेन्टेशन प्राविधिहरू प्रयोग गरी जाँच गरियो । डाटा विश्लेषणको लागि माइक्रोसफ्ट एक्सेल २००७ प्रयोग गरियो । १५० वटा दिशाको नमूनाहरू मध्ये ६६ वटा सकारात्मक पाइए, जसमा ४४% परजीवी संक्रमणको समग्र व्यापकता देखियो । रिङ-नेक्ड कालिजहरूमा ५ विभिन्न परजीवी वर्गहरूको पहिचान गरियो । नेमाटोडहरूमा *Heterakis* spp= (१२.६६%), *Capillaria* spp= (८%), *Ascaridia* spp= (६%) र *Strongyle* spp. (५.३३%) प्रयोगशालामा परिक्षण गर्दा देखिए । प्रोटोजोआमा केवल *Eimeria* spp= (८.६६%) मात्र पाइएको थियो । अध्ययनले देखाए अनुसार भाले कालिजहरूमा (४९.३%) पोथीहरू भन्दा (३८.६६%) उच्च परजीवी संक्रमण थियो । तर, लिङ्गको आधारमा परजीवी संक्रमणको फरकलाई संख्यागत रूपमा अर्थपूर्ण पाइएन (p=०.२६) । यस अध्ययनले कम उचाइमा (४८%) र उच्च उचाइमा (३६%) सबैभन्दा कम परजीवी संक्रमणको प्रसार देखायो, तर कम उचाइ र उच्च उचाइको बीचमा कुनै संख्यागत रूपमा अर्थपूर्ण फरक देखिएन (p=०.२२२) । भाले र पोथी कालिजहरूमा समान परजीवीहरूको उपस्थितिहुनुको कारण तिनीहरूको समान मौसम, खाद्य स्रोतहरू र वातावरण हुनसक्छ ।

यो अध्ययनले दाङ उपत्यकाका रिङ-नेक्ड कालिजहरू पेटको परजीवीहरूबाट प्रभावित हुने सम्भावना बढी हुने देखाएको छ , जुन व्यवस्थापन अभ्यासहरू र रोग नियन्त्रणको लागि आधारभूत डाटा हो ।

Table of contents

List of tables

List of figures

List of photographs

List of abbreviations

Abbreviated form	Details of abbreviations
CDZ	Central Department of Zoology
df	Degree of freedom
GI	Gastrointestinal
i.e.	That is
IUCN	International Union for Conservation of Nature
NaCl	Sodium Chloride
P value	Probability value
rpm	Rotation per Minute
TU	Tribhuvan university
w/v	weight/volume
µm	Micrometer
χ^2	Chi square

1. Introduction

1.1 Background

Pheasants are the birds that belong to different genera within the Phasianinae subfamily, which is a part of the Phasianidae family in Galliformes order. Although they have been introduced all over the world, pheasants are indigenous to Asia. Out of the 181 pheasant species, 49 are native to Asia (Poudel et al., 2023). Nepal is home to eight species of pheasants. These include highland species such as the Himalayan monal, satyr tragopan, blood pheasant, koklass pheasant, and kalij pheasant. In addition two lowland species such as red junglefowl and blue peafowl have also been recorded in the country (Poudyal et al., 2011). In Europe and parts of the Eastern world, pheasants are mainly kept as game birds for hunting. However, in South Asia native pheasant species are listed by the International union for Conservation of Nature (IUCN) due to their conservation concerns. Pheasants are large ground-dwelling birds that can be found all over Nepal, from lowland to high alpine areas. Some species, like the Himalayan Monal, which is national bird of Nepal, prefer high altitude rhododendron forests while others like Kalij pheasant thrive in lower elevation woodlands. Among these, the ring necked pheasant (*Phasianus colchicus*) found in Germany is considered a hybrid of different subspecies of *Ph. c. torquatus* and *Ph. c. mongolicus* which is distinguished by its characteristic white neck feathers(Curland et al., 2018). It was originally found from the Black Sea region across the dry land of Central Asia, extending eastward to South Korea and Siberia. They mostly favors structurally semi-open territory with cover from trees and hedges, while it is also inhibit nearby reedy and sparse woodland region (Liebing et al., 2020). Adult pheasants mainly feed on plant based materials, including seeds, berries, tubers, root shoots, and fresh green sprouts. In Nepal ring necked pheasant can be included in poultry too. Poultry is one of the most rapidly expanding sectors within Nepal's agriculture industry that contribute approximately 4% to the country's national GDP (Nirmal & Pokharel, 2017). The farming of it in Nepal has great potential for meat, eggs and feathers. They are well-known for their nutritious meat, which is low in fat and rich in essential amino acids. They are also easily recognizable where males are larger with colorful plumage while females are simple (Ashraf, 2015). Pheasant rearing mainly includes two methods: intensive and semi-intensive, with the semi-extensive system practiced in Europe. The semi-intensive method is also called the semi-natural rearing system(Gheța et al., 2020).

Poultry viruses can also affect the pheasants that belong to the order Galliformes. Transmission of viral diseases such as Newcastle diseases, infectious laryngotracheitis and infectious bronchitis that affecting pheasant is believed to occur through either direct or indirect contact with domestic birds or wild birds carrier (Curland et al., 2018). Gastrointestinal (GI) parasites are the most common infectious agents that infect the digestive tract of humans and animals including the domesticated and wild birds. The gastrointestinal tract of birds usually contain a variety of parasites, and the impact of these parasites varies depending on the hosts health and environmental factors (Carrera-Játiva et al., 2018). Roundworm (*Heterakis isolonche* , *Syngamus trachea*, *Ascaridia* spp. and *Capillaria* spp.) and coccidian (*Eimeria* spp.) are the major pathogens that are common in both wild and farmed game birds which are known to cause gastrointestinal disturbance and reduce breeding rates (Noor et al., 2024). Nematodes represent the most important group of helminth parasites in poultry, both in terms of species diversity and the extent of damage they inflict. The primary genera are *Capillaria*, *Ascaridia* and *Heterakis*. Infection with these gastrointestinal parasites leads to stunted growth, deteriorating health, decreased egg production, loss of appetite, altered vocalization, respiratory issues, weight loss, bloody diarrhea and increased mortality (Lapang et al., 2022).The prevalence and severity of helminth infections can be affected by various factors such as bird's age, sex and breed. Likewise, the dynamics of the parasite population may be changed by environmental conditions, that leads to major variation in the occurrence and severity of helminth infections (Jegede et al., 2016). Especially warm and moist soil can act as a key reservoir and transmission site for the external larval stages of helminthes. High temperature and to some extent humidity promote the proliferation of numerous insects that can serve as vectors for helminthes. These environmental conditions may play a role in the widespread presence and variety of nematode and cestode species in poultry, particularly during the tropical rainy season (Dube et al., 2010).

1.2 Statement of problem

The ring necked pheasant (*Phasianus colchius*) is a widely distributed as game bird species that plays a vital ecological and economic role in different region. Despites its importance, there is limited scientific information available regarding the health status of these birds. GI parasitism can significantly affect the health; reproduction and survival of avian species that leads to population decline and affect biodiversity. In Dang Valley the ring necked pheasant may be exposed to various environmental factors and the risk of parasitic infection

is potentially high. The prevalence, diversity and pathogenic impacts of GI parasites in ring necked pheasants in Dang Valley remains undocumented. So the lack of such data hampers the development of effective health monitoring and conservation strategies for this bird species. This study aims to fill the knowledge gap by identifying the GI parasites present in ring necked pheasants of Dang Valley. The understanding of the parasitic burden is crucial for improving the management, conservation and wellbeing.

1.3 Objectives

1.3.1 General objective

To determine the prevalence of gastrointestinal parasites of ring necked pheasants in Dang Valley, Nepal.

1.3.2 Specific objectives

- To identify the prevalence of gastrointestinal parasites of ring necked pheasants based on species and sex.
- To identify the prevalence of gastrointestinal parasites of ring necked pheasants across different farms and various altitudes within the Dang district.

1.4 Research questions

- What is the overall prevalence of gastrointestinal parasites in ring necked pheasants in the Dang Valley?
- What are the various types of gastrointestinal parasites found in ring necked pheasants of the Dang Valley?
- Is there any difference in gastrointestinal parasites based on sex and altitude in ring necked pheasants of the Dang Valley?

1.5 Significance of the study

The study of gastrointestinal (GI) parasites in farm pheasants in Dang district, Nepal is significant for improving poultry health, economic stability, and public safety. GI parasites can cause poor growth, reduced egg production, and even mortality in pheasants that directly impact the farm productivity. As pheasants farming are an emerging business in Nepal, uncontrolled parasitic infections can lead to economic losses due to increased treatment costs and reduced market value of infected birds. Similarly, some GI parasites are zoonotic, posing potential public health risk. By identifying specific parasites, it helps

to recommend targeted deworming and biosecurity measure, reducing drug resistance and environmental contamination. Furthermore, this research contributes to parasitological studies, as limited data exist on pheasant parasites in Nepal. These findings can help in formulating government guidelines for parasites control and raising awareness among farmers about best management practices. Ultimately, this study is essential for enhancing pheasant farming, ensuring economic benefits, and safeguarding both animal and public health.

2. Literature Review

The ring-necked pheasant (*Phasianus colchicus*) is a widely distributed as game bird, but it is raised for meat, eggs and feathers in some parts of Nepal. Despite being less common than chicken, pheasant are increasingly farmed as alterative poultry species due to their unique meat quality, economic potential and adaptability. Research on gastrointestinal parasites in ring necked pheasant within Nepal is limited. However, studies from nearby regions and on other avian species in Nepal provide insights into potential parasitic challenges these birds may face. So some of the important literature related to poultry birds has been reviewed here.

2.1 In the global context

A study conducted by (Laatamna et al., 2019) investigated the occurrence and variety of gastrointestinal parasites in both captive and domestic birds found in zoological parks and rural regions of Algeria. They collected 84 fresh fecal samples from 11 birds where they observed 19% prevalence of gastrointestinal parasite. The study reported a prevalence of 6% for *Cryptosporidium*, 4.8% for *Eimeria*, 3.6% for *Capillaria*, and 4.8% for *strongyle* larvae. The distribution of these parasites varied across different bird species. In Nigeria, a higher GI prevalence of 95.65% from Ilorin was reported and the recorded parasites were *Eimeria* spp., *Ascaridia galli*, *Heterakis gallinarum*, *Subulura brumpti* and *Capillaria annulata* which was performed by direct wet mount examination and floatation techniques (Ola-Fadunsin et al., 2019). A similar study was conducted by (Jegede et al., 2019) reported 95 % parasitic prevalence and found 16 different species of parasites like *Ascaridia* sp., *Strongyloides* sp., *Capillaria* sp., *Heterakis* sp., *Tetrameres* sp., *Spirurid* sp., *Raillientina* sp., *Davainea* sp., *Subulura* sp., *Oxyuris* sp., *Cyathostoma* sp., *Syngamus* sp., *Eimeria* sp., *Cryptosporidium* sp., *Sarcocystis* sp., and *Trichomonas* sp. in Gwagwalada. A survey by (Akram et al., 2019) in Gujranwala and Jhang districts measure the prevalence of gastrointestinal parasites in captive birds. The study examined 613 bird samples from 19 different species and found a parasite prevalence of 54.32%. (Ibrahim et al., 2006) studied 100 fecal samples from ostriches in a semi-arid area of northeastern Nigeria, 50 of which were taken from the birds in the wild and 50 from captivity. Using the method concentration, along with several helminth parasites like *Ascaridia* spp., *Strongyloides* spp., and *Capillaria* spp. a singles protozoan parasite *Eimeria* spp. was discovered. The average number of ostriches with gastrointestinal parasites was 36 (72%) in the free-living

population and 44 (88%) in the captive population. (Wozerou, 2014) conducted a survey to assess a prevalence of GI parasites of Scavenging chickens in Foka Division, South West Cameroon. A total of 889 fecal samples were examined by using salt/sugar floatation techniques, among them 500 were infected with the prevalence values of *Eimeria* 33.9%, *Ascaridia galli* 14.3%, *Heterakis gallinarium* 9.8% *Strongyloides* spps. 8.6% *Capillaria* spps. 5.7% and *Trichuris* 74%. A study by (Sreedevi et al., 2016) conducted on desi fowl (*Gallus gallus domesticus*) in and nearby village of Gannavaram, Andra Pradesh, India found a 63.21% prevalence of gastrointestinal parasites. The data showed that Desi fowl had the highest prevalence of gastrointestinal parasites during rainy season (43.41%), followed by the summer season (38.91%) and the winter season (17.68%). However, infections with *A. galli* and *C. annulata* were more prevalent during the summer. In this study, the authors emphasize the necessity of enhancing backward poultry management practices to reduce the impact of parasitic infections on birds. In order to determine the GI helminthes infection in waterfowl (Padilla-aguilar et al., 2020) imported 200 digestive tracts where 23 helminths were identified among them six trematodes, four cestodes, eleven nematodes and two were acanthocephalans were found. Symeonidou et al.(2019) examined 141 clinically healthy ostriches from four different regions in Greece and found that protozoan infections had the highest prevalence at 65.9%. Among the samples, *Entamoeba struthionis* cysts were identified in 57.4% and *Balantioides coli* cysts in 39%. *Cryptosporidium* oocysts were detected at a lower rate of 2.1%. The study did not identify any helminth infections. At the Zoo Park of the Federal University of Agriculture, five types of gastrointestinal parasites were identified: *Ascaridia*, *Ascaris*, *Capillaria*, *Strongyloides*, and *Rallietina*. During various weeks of sample collection, three of these parasites were detected in ostriches, owls, and mallard ducks. Both ostriches and owls exhibited cases of mixed infections. Among all parasites, *Capillaria* species showed the highest occurrence, being present in 57.1% of the cases (Sam-Wobo et al., 2017). Iraqi researchers (Alasadiy et al., 2022) found 65 pigeons i.e. 26% were infected from the 250 fecal samples of domestic and wild pigeons. The study identified three primary groups of parasites affecting birds: protozoa, including *Eimeria* spp., *Cryptosporidium* spp., and *Trichomonas gallinae*; cestodes, particularly *Raillietina tetragona*; and nematodes, such as *Ascaridia columbae*. The prevalence of GI parasites in domestic pigeon was higher than that of wild pigeon i.e., 32% and 20% respectively. According to the studies carried out by (da Silva et al., 2022) in the semiarid area of the Paraiba state of Brazil it was found that that nematodes and coccidian were found in 40.52% and 39.08% respectively in free-range

chicken. Mixed infections by nematodes and coccidian were observed in only 175 of the cases. However, nematodes belonging to the Heterakoidea superfamily and *Eimeria* species were found in all of the positive samples. The researchers concluded that nematodes and coccidia are highly prevalent in free-range chickens in Paraíba State, Brazil. Therefore, they recommended implementing proper sanitation and hygiene measure to enhance productivity by lowering the incidence of GI parasites.

In a study by (Aniefiok Udoh et al., 2014), a total of 196 turkeys, including 114 males and 82 females, were examined. The overall prevalence of gastrointestinal parasites was found to be 57.7%. The prevalence of GI parasites in males and females was 62.3% and 47.6% respectively. Likewise, (Hembram et al., 2015) reported that female *Banjara* fowls were more infected with GI helminthes than male *Banaraja* fowls. But the result conflicted with the research done by (Jegede et al., 2016) on local and exotic chicken which reported a higher prevalence of GI parasites in male chickens compared to female chickens. Similarly, a study performed by (Abdo Jilo et al., 2022) reported the higher prevalence of GI parasites in male turkey than the female ones.

Similarly, (Pariyar et al., 2021) investigated the prevalence of GI parasites in various captive mammals and pheasants at the high-altitude Padmaja Naidu Himalayan Zological Park in Darjelling hills. Using direct smear and concentration techniques, they analyzed 949 fecal samples, of which 222 (23.39%) tested positive for parasitic infections. The most commonly detected parasites were *Toxocara* spp., *Eimeria* spp., *Isopora* spp., and *Ascarid* spp. A similar study conducted on wild and captive birds at Pakistan showed the more parasitic prevalence in low land areas in comparison of birds found in upland areas (Hafeez-ur-rehman & Hussain, 2023). But the study conducted by (Anggrahini et al., 2025) in three regions i.e. coastal, lowland and highland areas of Indonesia found the similar occurrence of infection in low land and highland areas (61.43% and 61.54%), while coastal areas had lower occurrence (30.30%).

2.2 Nepalese context

In Nepal, a lot of work has been done on gastrointestinal parasites in birds but in ring necked pheasant it was difficult to find so this study reviews the articles that are relevant to it.

According to (Adhikari et al., 2022), from the 155 fecal samples of household and temple pigeon 16 species of parasites including eight protozoan and eight helminth faunae were identified. The most frequently identified species included *Eimeria columbae* (46.5%),

Capillaria columbae (29.7%), *Eimeria columbarum* (23.2%), *Ascaridia* (22.6%), *Entamoeba* sp. (14.2%), and *Eimeria labeana* (10.3%). The study found that protozoa had a slightly higher prevalence (78.7%) compared to helminthes (69.7%). Temple pigeons hold the higher prevalence rate than the household pigeons (i.e. 95.6% vs. 75.4%). Similarly, (Jha et al., 2023) conducted a study on feral pigeons at two temples in the Kathmandu Valley and reported a 90.83% overall prevalence of gastrointestinal parasites. They identified one protozoan genus, *Eimeria* spp. (43.34%), and five helminth genera: *Capillaria* sp. (51.67%), *Ascaridia* sp. (27.50%), *Heterakis* sp. (19.17%), *Syngamus* sp. (4.17%), and *Tetrameres* sp. (1.70%). The prevalence of helminth parasites (83.34%) was found to be higher than that of protozoan parasites (43.34%). According to (Gurung & Subedi, 2018), out of 120 pigeon fecal samples collected from three temples in the Pokhara Valley, 69.16% (83 samples) tested positive for gastrointestinal parasite. A total of seven different parasites were identified, including one protozoan subclass, *Coccidia* (19.16%), and six genera of helminthes. Similarly, (Subedi et al., 2018) reported that chickens from the Lalitpur district were infected with one cestode, five nematode species, and four unidentified species. The highest prevalence was observed for *Heterakis gallinarum* (22.4%), followed by *Capillaria* spp. (16%), *Ascaridia galli* (10.4%), unidentified species (4.8%), and *Raillietina tetragona* (4%).

3. Materials and methods

3.1 Study area

The study was carried out in two sub metropolitian city (Ghorahi and Tulsipur Sub metropolitan) and one rural municipality (Banglachuli Gaupalika) of Dang district of Lumbini Province. It is located within the geographical coordinates of 28° 00' 0'' N to 82°15' 60''E at an altitude of 109 meters to 2914 meters above sea level. The total area of Dang is 2955 km^2. The three farms are within the distance of 25-30 km from each other. Among these three different farms of Dang district, Himalayan Agro farm and Chijman Pashupanchhi farm is located at low altitude within the elevation of approximately 700 m. from the sea level and Lekali farm is located at high altitude within the elevation of approximately 1550 m. from the sea level. A large number of people in this region are engaged in agriculture, animal husbandry and poultry farming on both small and large scale. Dang valley experiences a sub tropical climate with hot and dry during summer, heavy rainfall during the monsoon and cooler night and occasional morning fog during the winter season.

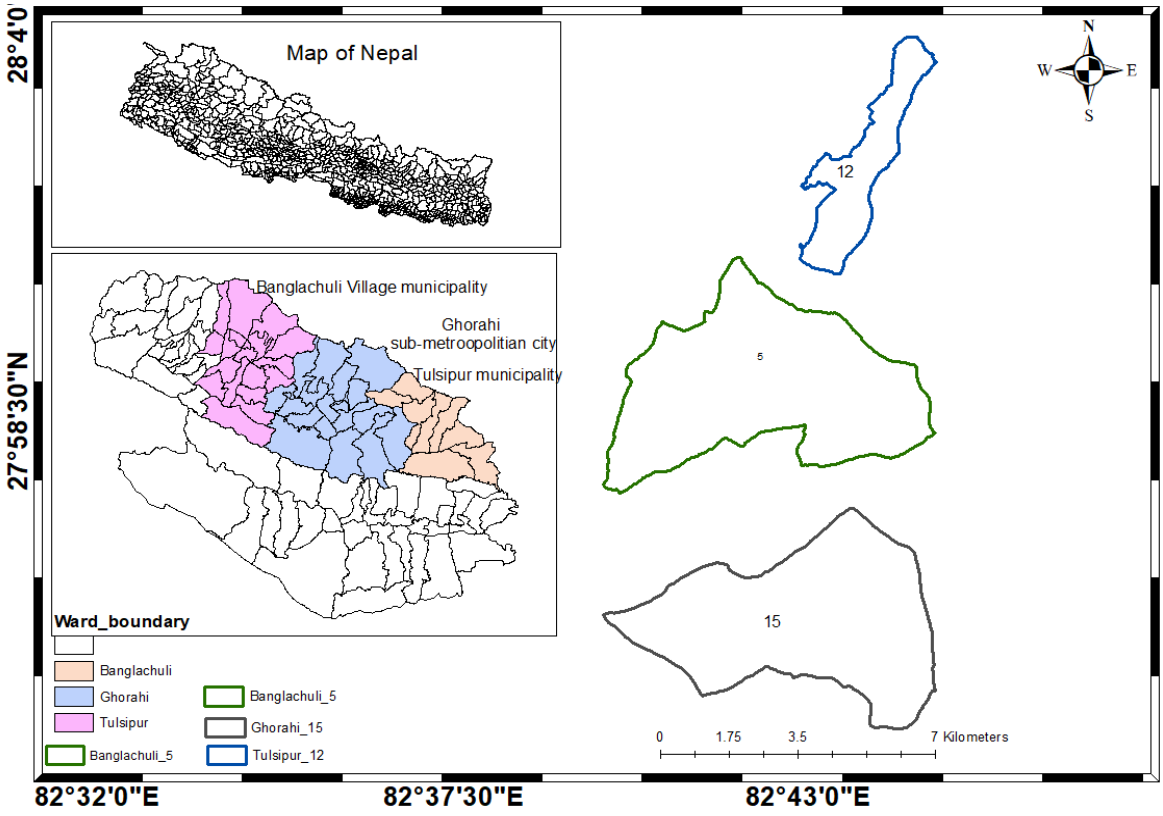

Figure 1: Map of Nepal showing the study area

3.2 Methods

3.2.1 Study process

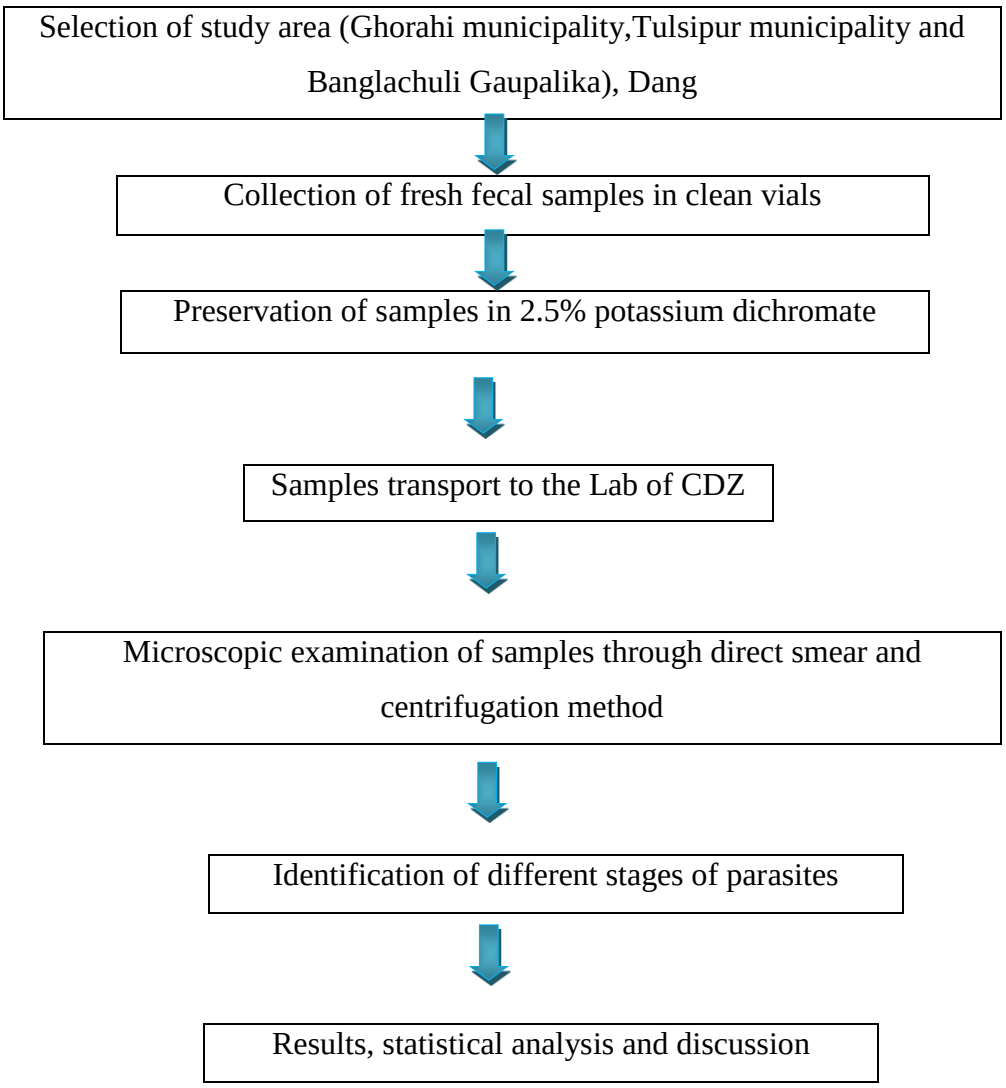

Figure 2: Flow chart showing study process

3.2.2 Study period

The study was carried out during the month of November, 2024.

3.2.3 Sample size

150 total fecal samples were collected from the three different farms of Dang district during the month of November, 2024. Among them 75 samples were from male and 75 samples were from female. The highest number of pheasant was 400-500 found in Himalayan Agro farm, followed by Lekali farm with 300 and Chijman Pashupanchhi farm with 200-250.

3.2.4 Sample collection and preservation

The ring necked pheasant's fresh fecal samples were collected early in the morning. Samples were collected on the site over the period of seven days. With the help of farm worker, ten to fifteen grams of feces were gathered using a plastic spoon in a sterile clean vial. At first a fruit basket was made ready by placing newspaper or polythene on it. Then the pheasant was kept in fruit basket until it defecate on the newspaper or polythene and then it was released by marking a mark on its leg for not repeating themselves. The feces that had been collected on the newspaper or plastic was then transferred to a sterilized clean vial containing a solution of 2.5% potassium dichromate ($K_2Cr_2O_7$) and labeled. The sex of the pheasant was distinguished by their morphological traits and the samples were transported safely to the lab of Central Department of Zoology. The samples were preserved in the refrigerator at 4˚C

3.3 Laboratory examination

The collected fecal samples were transported to the lab of the Central Department of Zoology, Kirtipur, Kathmandu for analysis. Under a microscope, the samples were examined for different stages of parasites using stained smear and concentration methods such as flotation and sedimentation(Zajac et al. 2012).

3.3.1 Direct smear method

1-2 drops of fecal samples were mixed in a drop of normal saline on a clean glass slide using a clean toothpick and then covered with a fresh cover slip. In the slide, a tiny drop of iodine was dropped. A smear was then made after the sample was placed over iodine. Under a microscope, the prepared smear was examined at two distinct magnifications: 100× and 400× respectively (Zajac et al. 2012)..

3.3.2 Concentration methods

Parasite eggs, cysts or larvae are usually visible in smears only during heavy infections. In most cases, they are hard to detect in direct smear mounts because of their low concentration in feces. As a results, flotation and sedimentation techniques were used to concentrate the samples (Zajac et al., 2012).

- **Floatation technique**

Approximately 2 grams of the fecal samples were thoroughly mixed with 12 ml of 0.9% (w/v) sodium chloride (NaCl) solution. The mixture was then filtered through a strainer into a 15 ml centrifuge tube. The filtrate was centrifuged at 1200 revolutions per minute (rpm) for 5 minutes. Following centrifugation, the supernatant was discarded, and the tube was filled with a 45% (w/v) NaCl solution. The sample underwent a second centrifugation at 1200 rpm for another five minutes. Afterward, the tube was slowly and completely filled with saturated NaCl solution until a convex meniscus formed at the top. A coverslip was gently placed on top of the tube and left undisturbed for ten minutes allowing the parasites eggs to float and stick to the coverslip. Finally, the coverslip was carefully removed and placed onto a glass slide for microscopic examination under a total magnification of 100× and 400× (Adhikari et al., 2021).

- **Sedimentation technique**

Approximately 2 grams of the fecal sample were combined with 12 milliliters of 0.9% (w/v) sodium chloride solution. The mixture was strained using a tea strainer, and the resulting filtrate was centrifuged at 1200 rpm for five minutes. After centrifugation, the supernatant was carefully poured off, and the tube was filled with 10 milliliters of 10% formalin and 3 milliliters of ethyl acetate. The sample underwent a second centrifugation at the same speed and duration. Following this, the supernatant was discarded, and the remaining sediment was examined microscopically at total magnifications of 100× and 400× (Adhikari et al., 2021).

3.3.3 Identification of eggs and cysts of parasites

The identification of eggs and cysts of parasites were confirmed by comparing with reference images or keys of published literature, journals and books (Zajac et al., 2012).

3.3.4 Data analysis

The data that were collected from laboratory tests were first organized and analyzed using Microsoft Excel. Percentages were calculated to find out the prevalence of different conditions. Pie charts and bar graphs were used to clearly show the results and make comparisons easier. Chi-square test was performed by using R-Studio for statistical analysis of data. A result was considered statistically significant if the p-value < 0.05, and all the results were checked within a 95% confidence level.

4. Results

4.1. Overall prevalence of GI parasites

Out of a total of 150 fecal samples analyzed, 66 were tested positive for gastrointestinal parasites. It indicates a 44% of overall prevalence rate of parasitic infection. The remaining 84 samples were tested negative (Figure 3).

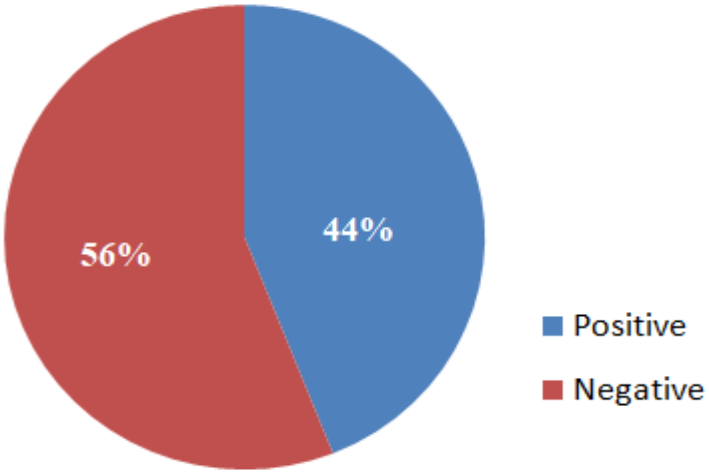

Figure 3: Overall prevalence of GI parasites in ring necked pheasant

4.2 Species wise prevalence of parasites of different forms

Fecal samples were collected from three poultry farms (Lekali poultry farm, Himalayan Agro farm and Chijman Pashupanchhi farm) with 50 samples obtained from each. Through fecal analysis, five different parasitic species were identified. Among them, only one protozoan species, *Eimeria* spp., was detected in 18 samples (12%). The remaining four were helminth species, with *Heterakis* spp. being the most prevalent found in 19 samples (12.66%). This is followed by *Capillaria* spp. in 12 samples (8%), *Ascaridia* spp. in 9 samples (6%), *Strongyle* spp. in 8 samples (5.33%) (Table 1).

Table 1: Species wise prevalence of parasites of different farms

S.N	**Class**	**Species**	**Name of farms**			
			Lekali Poultry farm (n=50)	Himalayan Agro farm (n=50)	Chijman Pashupanchhi (n=50)	**Prevalence rate (%)**
1.	Protozoa	*Eimeria* spp. (n=18)	5 (10%)	6(12%)	7(14%)	12%
2.	Nematodes	*Heterakis* spp. (n=19)	6 (12%)	8(16%)	5(10%)	12.66%
		Capillaria spp. (n=12)	4(8%)	5(10%)	3(6%)	8%
		Ascardia spp. (n=9)	3 (6%)	4(8%)	2(4%)	6%
		Strongyle spp. (n=8)	-	5(10%)	3 (6%)	5.33%

4.3 Sex wise prevalence

Out of 150 total fecal samples collected, an equal number of samples 75 each from males and female were taken for analysis. Among these, 29 samples (39%) from males and 37 samples (49%) from females were tested positive for gastrointestinal parasites (Figure 4). This indicates a higher prevalence of GI parasites in females as compared to males. However, statistical analysis revealed that the difference in infection rates between sexes was not significant (χ^2=1.325, df=1, p=0.26).

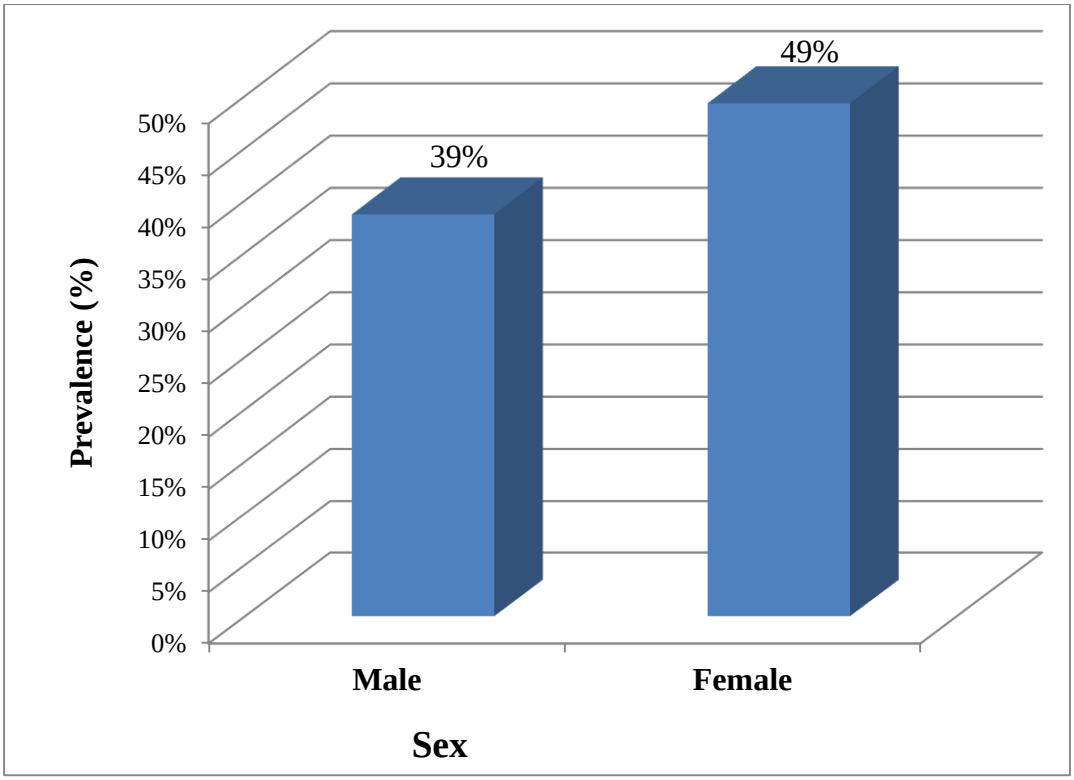

Figure 4: Bar graph showing prevalence of GI parasites in different sex group

4.4 Altitude wise prevalence

The prevalence of infection with different intestinal parasites for low altitude (700 m) and high altitude (1550 m) is shown in the table. Out of 100 fecal samples collected from low altitude area, 48 samples i.e. 48% were positive for at least one parasite. Similarly, of the 50 fecal samples collected from high altitude, 18 fecal samples i.e. 36% were positive for at least one parasite (Table 2). Statistical analysis indicated that the difference in infection rates between the two altitude was not significant (χ^2=1.49, df =1, p=0.222).

Table 2: Altitude wise prevalence of GI parasites

	Altitude wise			
	Examined	**Positive**	**Prevalence**	**p-value**
Low altitude (~700m)	100	48	48%	0.222
High altitude (~1550m)	50	18	36%	

4.5 Farm wise prevalence of GI parasites

For the prevalence of GI parasites, a total of 150 samples were collected from three farms with 50 samples taken from each farm. Among them, Himalayan Agro Farm showed the highest prevalence, with 28 samples (56%) positive. This was followed by Chijman

Pashupanchhi Farm 20 samples (40%) and Lekali Poultry Farm with 18 samples (36%) positive (Table 3). Statistically, the difference in prevalence of GI parasitic infection amongst farms was found to be insignificant (χ^2=4.5455, df=2 p=0.103).

Table 3: Farm wise prevalence of GI parasites

	Farm wise			
	Examined	**Positive**	**Prevalence**	**p-value**
Lekali Farm	50	18	36%	
Himalayan Agro Farm	50	28	56%	0.103
Chijman Pashupanchhi Farm	50	20	40%	

4. Discussion

The ring necked pheasant (*Phasianus colchius*) is one of the most widely recognized and economically important game birds in the world. They are native to Asia and have been introduced to numerous country including North America and Europe where it thrives in diverse habitats ranging from farmlands to grasslands and forest edges. Ring necked pheasants in Nepal, including regions like Dang valley, are susceptible to various gastrointestinal parasites that can impact their health and survival. While specific studies focusing on ring necked pheasant in Nepal are limited, so research from other areas provides valuable insights into the parasitic challenges these birds may face. The main gastrointestinal parasites that are reported by many research are, *Heterakis* spp., *Ascaridia* spp., *Eimeria* spp., *Capillaria* spp., *Coccidia* oocyst spp., *Raillietina* spp., *Taenia* spp., etc.

This research was done in order to study the gastrointestinal parasites in ring necked pheasants of farms of three different municipality of Dang district. The current study recorded a 44% prevalence of gastrointestinal parasites in ring-necked pheasants. This finding is consistent with the 48.11% prevalence reported by (Patel et al., 2000) in a study conducted on various wild bird species at the Kamal Nehru Zoo in Gujarat. The findings of the present study are also largely in agreement with those of previous researchers, such as (Ilić et al., 2018) who reported a 51.4% prevalence rate in birds from a zoological garden in Serbia and (Akram et al., 2019) who observed a 54% prevalence in captive birds in Pakistan. In the current study, one protozoan species and four helminth species were identified. This is comparable to the findings of (KÖSE et al., 2009) who also reported five helminth species, four nematodes and one cestode with to (Chaudhary & Pandey, 2019) who identified five nematode species. The protozoa: *Eimeria* and the helminthes: *Capillaria* spp., *Heterakis* spp., *Ascardia* spp. and *Strongyloides* spp. infection known to occur in this study also infected the free ranging Peacocks in Tamil Nadu, India (Kathiravan, et al., 2017), captive birds in Serbia (Ilić et al., 2018), domestic Turkey in Nigeria (Aniefiok Udoh et al., 2014) and pigeon in central Nepal (Adhikari et al., 2022). Such types of infection may affect the growth; reduce in production of eggs, weight loss, loss of appetite, bloody diarrhea and change in vocalization.

By using the direct smear and concentration techniques (floatation and sedimentation techniques) this study identified one protozoan (*Eimeria* spp.) and four helminth parasites. *Eimeria* spp. is recognized as the causative agent of coccidiosis in birds, a common protozoan disease that is emerging as a growing issue in poultry farming worldwide. In this

study, the overall prevalence of *Eimeria* spp. was 12%, which is lower than the rates reported in scavenging chickens from the semi-arid regions of Eastern Kenya by (Mungube et al., 2008) and in domestic poultry from Colombia (Montes-Vergara et al., 2021). These variations may be due to the differences in management practices, climatic conditions, bird age, and the presence of intermediate hosts. The study conducted by (Luján et al., 2008) in Peru, *Capillaria* spp. (9.70%) which is comparable to those found in the current study. Similarly, the study done by (Aniefiok Udoh et al., 2014) on Turkey in Nigeria, found the lower prevalence rates than the current study. Similarly, studies conducted by (Sadaf et al., 2023) and (Stapf et al., 2024) reported higher prevalence rates of *Capillaria* spp. compared to the current findings Additionally, *Ascaridia* spp. showed a slightly higher prevalence of 17.2% in Nigeria, as reported by (Rufai & Jato, 2017), than observed in this study. These differences may be due to the factors such as sample size, methodological approaches, management systems, hygiene conditions, use of anti-helminthic treatments, and the timing of sample collection.

In the present study, the prevalence of parasitic infection was higher in female birds (49.3%) compared to males (38.66%), aligning with findings from a previous study on game birds in Bangladesh (Hilmi et al., 2018). (Hembram et al., 2015) also reported that male birds tend to show greater resistance to parasitic infections than females, which supports the current results. Likewise, the prevalence of intestinal parasites was slightly higher at lower altitudes than at higher elevations, consistent with observations from a study conducted in the different altitudes in Punjab Pakistan. This findings indicated that lower temperatures at higher elevations can reduce the development of parasites that leads to lower parasite prevalence and higher in temperature in low altitude provide favorable conditions for parasite growth and transmission (Hafeez-ur-rehman & Hussain, 2023). These findings suggest that the rising temperatures and humidity due to climate change are likely to increase parasite prevalence in birds.

The prevalence rate of 12.66% *Heterakis* spp, 8% *Capillaria* spp., 6% *Ascardia* spp and 5.33% *Strongyle* spp.(5.33) in the current study were likely comparable to those found in earlier study (Adhikari et al., 2022) for *Heterakis* spp. (7.1%), *Strongyle* (5.2%), *Capillaria* spp. (5.8%). The Rupandehi report identified *Ascaris* spp. (15%) and *Heterakis* spp. (5%) as the most common helminth parasites, that is consistent with the current study, which observed similar prevalence rates. In the current study, the overall prevalence of parasitic infections in the birds was found to be 44%. This rate is comparatively lower than other

studies conducted in Nepal. For instance, a study on pigeons in Nepal reported a much higher prevalence of 69.16%. Additionally, research from central Nepal involving household and temple pigeons found an even higher prevalence rate of 87.1%. The variations in prevalence rates may be due to the differences in geographical locations, bird species, management practices, environmental conditions, and the types of parasites present in the respective regions. The lower prevalence found in the present study may also reflect regional differences or variations in the study design and sample size.

5. Conclusions and recommendations

5.1 Conclusions

The study was carried out to find the GI parasites of ring necked pheasants in Dang Valley. 150 total fecal samples were collected from the three different farms of Dang Valley. The study revealed a prevalence (44%) of gastrointestinal parasites in ring necked pheasants of Dang Valley. Among the parasites identified, *Heterakis* spp. was the most prevalent helminth, while *Eimeria* spp. was the only protozoan detected. Sex wise analysis indicated a higher prevalence in females (49.3%) compared to males (38.66%), though this difference was statistically insignificant. Similarly, altitude-wise comparison showed a higher infection rate at lower altitudes (48%) than at higher altitudes (36%), but the variations was not statistically significant.

Overall, the results showed that the ring necked pheasants have a variety of gastrointestinal parasites in Dang Valley. This highlights the need of better parasite management methods, deworming and improved farm hygiene practices.

5.2 Recommendations

- Routine fecal examination and regular deworming should be carried out.
- Regularly clean and disinfect housing, feeders and waterers should be used.
- Anticoccidial medications and coccidiosis vaccination programs should be implemented.
- Rotate pens and avoid overcrowding to reduce fecal contamination.

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Appendix I (Photographs)

Eggs of parasites in Ring-necked Pheasant under 100 X × 400 X microscopes

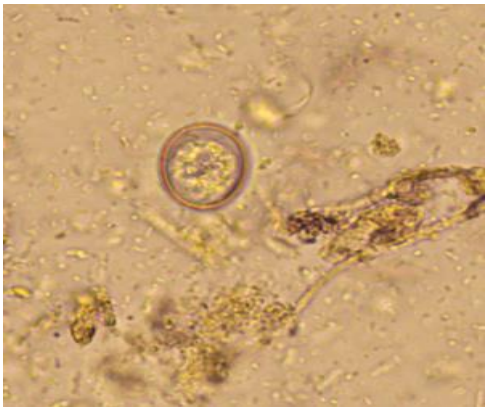

Photo 1: *Eimeria* spp. egg

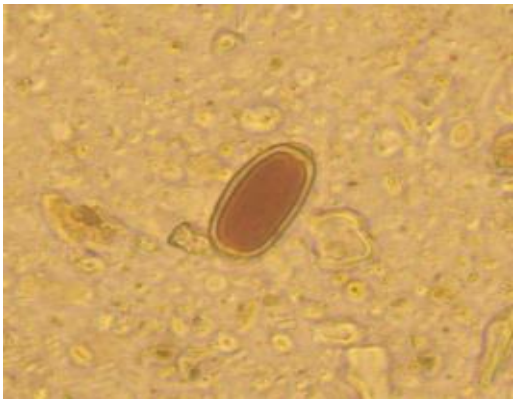

Photo 2: *Capillaria* spp. egg

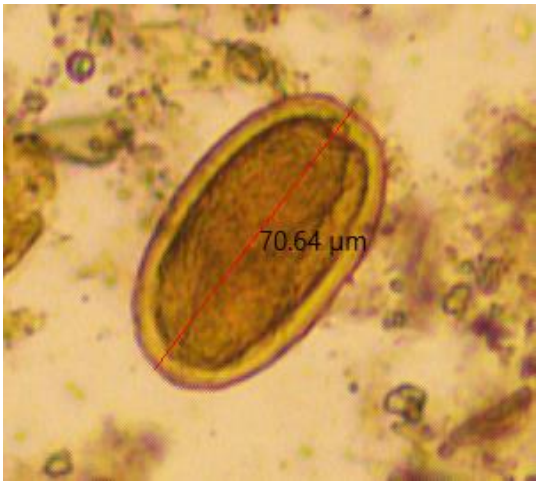

Photo 3 : *Heterakis* spp. egg

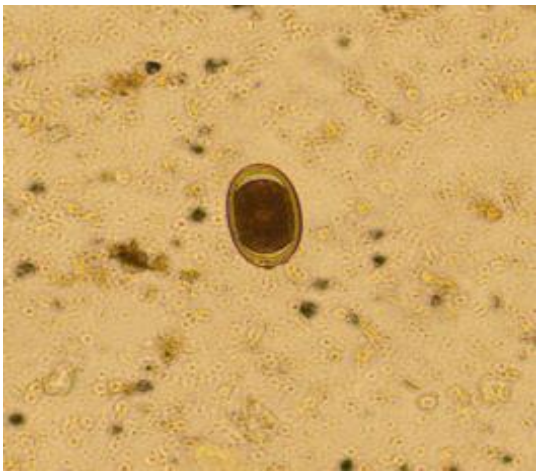

Photo 4 : *Ascaridia* spp. egg

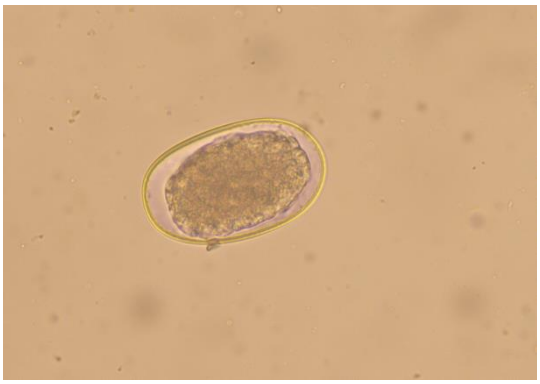

Photo 5 : *Strongyle* spp. egg

Appendix II (Photographs)

Photo 7: Farm worker working in the farm

Photo 8: Pheasant farm

Photo 9: Using fruit basket for sample collection

Photo 10: Marking pheasant for not repetition

Photo 11: Microscopic examination

Appendix III Materials Required

Materials for field

i. Sterile vials iv. Plastic spoon

ii. Gloves v. Mask

iii. Transparent plastic/ Newspaper

Materials for Laboratory

i. Gloves and mask ix. Cover slips

ii. Spatula x. Petri disc

iii. Beaker xi. Tea strainer

iv. Measuring cylinder xii. Cotton bud

v. Toothpicks xiii. Centrifuge tube

vi. Test-tube stand xiv. Centrifuge machine

vii. Droppers xv. Refrigerator

viii. Glass slides xvi. Electric microscope

Chemicals

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

ii. Distilled water v. Methylene blue

iii. NaCl solution