FREQUENCY OF GASTROINTESTINAL PARASITES OF INDIAN SPOTTED EAGLE (*Aquila hastata,* Jacques Brisson 1760) IN LUMBINI AND KOSHI TAPPU, NEPAL

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Submitted to

Central Department of Zoology

Institute of Science and technology

Tribhuvan University

Kathmandu Nepal

March, 2023

DECLARATION

I hereby declare that the work presented in this thesis entitled "**Frequency of gastrointestinal parasites of Indian Spotted Eagle (*Aquila hastata*) in Lumbini and Koshi Tappu of 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 thesis entitled "**Frequency of gastrointestinal parasites of Indian Spotted Eagle (*Aquila hastata*) in Lumbini and Koshi Tappu of Nepal**" has been carried out by Ms. Linisha Gharti Magar for the partial fulfillment 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 thesis work has not been submitted for any other degree in any institutions.

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LETTER OF APPROVAL

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Ms. Linisha Gharti Magar entitled "**Frequency of gastrointestinal parasites of Indian Spotted Eagle (*Aquila hastata*) in Lumbini and Koshi Tappu of Nepal**" has been accepted as a partial fulfillment for the requirement of Master's Degree of Science in Zoology with special paper Parasitology.

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Linisha Gharti Magar

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CDZ	Central Department of Zoology
Df	Degree of Freedom
Fig.	Figure
GI	Gastrointestinal parasites
ISE	Indian Spotted Eagles
IUCN	International Union for Conservation of Nature
ML	Mililiter
MS Excel	Microsoft Excel
NaCl	Sodium Chloride
P value	Probability Value
RPM	Revolutions Per Minute
sp.	Species
X^2	Chi-squre

ABSTRACT

Indian Spotted Eagles are a vulnerable species and their population is declining due to various parasitic factors. Parasitic infections in wild birds, especially migratory ones, can affect their health and population dynamics. The objective of this study was to find the frequency and intensity of gastrointestinal parasites in Indian Spotted Eagle (*Aquila hastata*) in Lumbini and Koshi Tappu of Nepal. Fresh fecal samples were collected during the breeding season in Lumbini and Koshi tappu in 2019, and samples were collected near the nesting tree. The collected fecal samples were processed and examined using direct microscopic examination, flotation technique, and sedimentation and concentration methods. A total of 40 fecal samples were analyzed and only 13 were positive for gastrointestinal parasites. The overall frequency of gastrointestinal parasites in Indian Spotted Eagles was 32.5%, and *Neodiplostomum* sp. is the most prevalent parasite. The Indian Spotted Eagle is infected with parasites, including helminths and protozoa. Lumbini had higher parasitic infections than in Koshi tappu. The frequency of parasites in the two locations did not differ significantly. Additionally, Indian Spotted Eagles displayed a variety of infections, including combinations of specific infections and mixed infections. The highest level of infection was found in *Neodiplostomum* sp. followed by *Strongyle* sp. and *Nematostrigea* sp. followed by *Strigea* sp. and *Isospora* sp. The results of this study suggest that Indian Spotted Eagles should be monitored regularly for parasitic infections including *Neodiplostomum* sp. Conservation efforts should also focus on habitat preservation and ecological balance to reduce parasitic infections risk in Indian Spotted Eagles.

CHAPTER 1: INTRODUCTION

1.1 Background

Indian Spotted Eagles (*Aquila hastata*) are widely distributed species found in Pakistan, Nepal, India, and Myanmar's lowlands; they have been recorded in very low densities in these areas (Sant *et al.* 2013, Piratelli *et al.* 2015). Indian spotted eagles (ISE) measure approximately 60 cm in length. There is a broad head, a wide mouth, and round nostrils on this species. Generally brown in color, it can be identified easily (Shivaprakash *et al*. 2006, Inskipp *et al*. 2013, Sant *et al*. 2013). Indian Spotted Eagles are distinguished by their spotted plumage and powerful talons for hunting small mammals, reptiles, and birds (Inskipp *et al*. 2013, Sant *et al*. 2013, Chandrakar & Dhuria 2021). In addition to being top predators, they also play a crucial role in maintaining the ecological balance of ecosystems (Dravecký *et al*. 2008, Vali & Magalhaes 2022). Besides these, humans and other animals rely on birds for food and pollination, which is important to economic sustainability and the health of our environment (Ombugadu 2018). Globally, Indian Spotted Eagles is a vulnerable species and the population of the species is declining due to habitat loss and degradation, as well as hunting and trapping for trade (Inskipp *et al*. 2013). In addition to these, they are susceptible to various diseases, climate change, and invasions of species, pollution, and anthropogenic activities. As a result, their population, timing of migration, breeding, health, and reproduction can be affected (Crick 2004, Ferket & Gernat 2006, Robinson *et al*. 2010, Piratelli *et al*. 2015). Wildlife health complications and mortality are caused by parasitic infections and bird species conservation is affected due to parasites (Ombugadu 2018).

In the gastrointestinal tract of both wild and domestic birds, gastrointestinal parasites are a diverse group of organisms that exist as parasites (Laatamna *et al*. 2019). Among the animals that are parasites are protozoa, such as coccidia and cryptosporidia, as well as helminths, such as tapeworms and roundworms (Akram 2019). It is important to remember that there are a number of factors that influence the prevalence of gastrointestinal parasites in birds, including species, location, home range, behavior, and environmental conditions (Begum & Sehrin 2012, Laatamna *et al*. 2019). There is evidence to suggest that wild birds, especially those living near water sources and other birds, are more likely to be infected with parasites than domesticated birds (Akram 2019).

The health of birds and the dynamics of their populations can be greatly affected by gastrointestinal parasites (Begum & Sehrin 2012). It is common for infected birds to lose weight, have diarrhea, vomiting, and feel fatigued (El-Shahawy & Abou Elenien 2015). It is possible for these symptoms to lead to decreased fitness and increased mortality (El-Shahawy & Abou Elenien 2015). Furthermore, possible complications such as secondary infections can also be caused by gastrointestinal parasites (Begum & Sehrin 2012). Besides these, gastrointestinal parasites can have wider ecological implications beyond their direct impact on bird health. There may be a lower level of success for infected birds when it comes to foraging, reproducing, and defending their territories (Hussain *etal.* 2022). Ultimately, this can affect ecosystem functions and community structure.

1.2. Research objective

The general objective of this study was to find the frequency of gastrointestinal parasites in Indian Spotted Eagle (*Aquila hastata*) of Lumbini and Koshi Tappu of Nepal.

12.1 Specific objectives were

- To find out the frequency of gastrointestinal parasites of Indian Spotted Eagle.
- To find out the intensity of gastrointestinal parasites of Indian Spotted Eagle.

1.3. Significance of the study

The Indian Spotted Eagle is a South Asian bird of prey vulnerable species according to the IUCN red list of threatened species. The study will be very much beneficial for developing knowledge about parasitic infection of Indian spotted eagle in Lumbini and Koshi Tappu of Nepal. The result of the study will provide data of parasitic infection on Indian spotted eagle and help to know the frequency of gastrointestinal parasites on this species. In addition, the research will also provide necessary information related to Indian spotted eagle.

CHAPTER 2: LITERATURE REVIEW

A study conducted by (Laatamna *et al*. 2019) evaluated the prevalence and diversity of gastrointestinal parasites in captive and domestic birds in Algerian zoological parks and rural areas. The prevalence of gastrointestinal parasites was observed in 19% of 84 fresh fecal samples from 11 bird species. A prevalence rate of 6% was identified for *Cryptosporidium*, 4.8% for *Eimeria*, 3.6% for *Capillaria*, and 4.8% for strongyles larvae, respectively. There was a variation in parasite distribution among different species of birds. In addition, a survey was conducted in Gujranwala and Jhang districts to determine the prevalence of gastrointestinal parasites in captive birds. A total of 613 bird samples, belonging to 19 species, were examined, and a prevalence of 54.32% was revealed. A majority of the parasites were in the genera *Eimeria,* Ascaridia, *Capillaria*, and *Hymenolepsis*. It was found that age and rearing systems played a significant role in infection rates, with adult birds and those reared in aviaries most commonly infected (Akram 2019). Similarly, Bristol Zoo Gardens found gastrointestinal parasites in 31% of captive birds and 65.5% of free-ranging birds. It was found that captive birds were infected with ascarids, capillarids, oxyurids, strongyles, a trematode, and protozoans. Similar parasite types were present in free-ranging birds, such as ascarids, capillarids, oxyurids, strongyles, cestodes, trematodes, and protozoa. No significant difference was found between birds housed indoors and outdoors when it came to the prevalence and intensity of nematodes and coccidia. There is evidence that captive and free-ranging birds, especially closely related species, may share parasites (Carrera-Jativa *et al*. 2018). In addition, In Nigeria, gastrointestinal parasite profiles were determined among birds in zoological gardens. A total of 178 fecal samples were collected from 83 birds belonging to 14 species representing eight orders. Overall, 21.9% of animals tested positive for the disease, with Unilag Zoo having the highest prevalence. A total of five parasite species were identified, including two protozoa (Coccidian and *Balantidium* spp.) and three nematodes (*Capillaria* spp., Ascaris spp., and Strongyloides spp.). The most frequent parasite in the gastrointestinal tract was *Capillaria* spp. Mixed infections were observed in 10.1% of samples, and Strongyloides larvae were seen in 3.4%. Struthioniformes were most likely to be infected, with mixed infections occurring in every Anseriforme (Otegbade 2014).

Salem *et al*. (2022) conducted a study in Egypt to investigate the prevalence and intensity of external parasites in domestic pigeons, specifically focusing on deltamethrin's effectiveness as an insecticidal agent. They examined 300 pigeons, categorizing them into different age groups. In this study, 80.3% of pigeons were found to have external parasites. There were several parasite species identified, including *Pseudolynchi acanariensis*, *Hippobosca equina*, *Columbicola columbae*, *Menopon gallinae*, *Knemidocoptes* species, and *Dermanyssus gallinae*. There was a higher likelihood of infestation among squabs and young birds than among adults during spring and summer. Parasitic infestations were also analyzed with regard to oxidative stress parameters, such as the levels of serum zinc, serum malondialdehyde (MDA), and serum nitric oxide. Birds infected with the virus had reduced zinc levels in their serum, while MDA levels and serum nitric oxide levels increased. In order to manage infested birds and decrease external parasite incidence, the study recommends regular treatment with deltamethrin. Furthermore, to minimize external parasite infestations, pigeon management strategies must be implemented.

Badparva *et al*. (2015) conducted a cross-sectional study from December 2011 to December 2012, focusing on fecal samples from 451 birds, including hens, turkeys, sparrows, pigeons, and decorative birds. A total of 157 species of birds (34.8%) were found to be infected with intestinal parasites. A total of five protozoan species were identified, as well as two nematodes and two cestodes. The samples did not contain any trematodes. Among helminth parasites, *Raillietina* spp. (4.2%), and *Eimeria* spp. (7.1%), were the most common. In a study of the birds, 12 (2.7%) showed two mixed infections and 6 (1.3%) showed three mixed infections. Based on the study, western Iran birds have a high prevalence of intestinal parasitic infections. In order to assess the impact of these infections on bird mortality and performance, further research is needed. In Bangladesh, (Hoque *et al*. 2014) conducted a survey to assess the prevalence of gastrointestinal parasites in domestic and wild birds. Domestic ducks from indigenous populations suffered more parasitic infections during summer (39%) than during winter (22%). Domestic indigenous ducks and Muscovy ducks had multiple parasitic infections. Unlike domesticated and wild birds, most bird species and wild birds suffer from only one type of parasite infection. The most commonly detected parasite in domesticated and wild birds was Ascaridia spp., with an average egg load of 50-900**.** Both domesticated and wild birds were found to have Capillaria species and *Heterakis* species. In this study, the authors suggest that providing household farmers with education and improving bio security measures could mitigate parasitic infections impact on bird production.

The study conducted by (Ola-Fadunsin *et al.* 2019)in Nigeria examined several avian species for gastrointestinal parasites. Among the GI parasites detected in the study, *Eimeria* species, *Ascaridia galli*, and *Heterakis gallinum* were the most prevalent. In all avian species, multiple parasite co-infections were recorded, with pigeons and turkeys most commonly infected. There was a significant relationship between GI parasite infections and age, sex, and bird type. In order to implement preventive and control measures against GI parasites, it is important to understand their epidemiology. This is for maximum production and reproductive efficiency in the poultry industry. Furthermore, the prevalence and intensity of gastrointestinal parasites in birds kept at the University of Ilorin Zoological Garden in Nigeria. A total of 21 fecal samples were collected from nine birds. It was estimated that 71.4% of birds had gastrointestinal parasites, with the highest prevalence appearing in Ostriches, Crowned cranes, and African fish eagles. There was also a high prevalence of domestic pigeons and white-faced whistling ducks, while Marabou stock, white peafowl, and emus were less common. A total of four parasite species were identified, including two protozoans (*Coccidian* spp. and *Eimeria* spp.) and two nematodes (*Capillaria* spp. and *Ascaris* spp.). *Ascaridia galli* had the highest level of infection (358 eggs per gram), followed by *Capillaria* spp at 104 eggs per gram, and *Eimeria* oocysts at 70 eggs per gram. There is a risk to staff and visitors from parasites in the birds in the zoological garden, according to the study (Adeola *et al*. 2022).

Bayzid *et al*. (2023) studied the prevalence of helminth and protozoan infections in pet birds in Chattogram, Bangladesh. A total of 549 fecal samples and 311 blood samples were collected from a variety of pet birds. The study found helminth infestations to be 8.01% common, with nematodes ranking highest. A total of 11.1% of intestinal infections were caused by protozoa, mostly *Eimeria* species. There was a prevalence of 2.25% of haemoprotozoan parasites, with *Plasmodium* species being most prevalent. Similarly, (Begum & Sehrin 2012) surveyed 60 pigeons for helminth parasite infection. A total of 11 helminth parasite species were found infecting all birds. There were 4 trematoda species, 6 cestoda species, and 1 nematoda species in the sample. There was a slight higher level of infestation intensity among females than among males, and the highest level of infection intensity was observed in autumn. Additionally, parasites were not found in the oesophagus, crop, proventriculus, gizzard, gall bladder, liver, kidney, or muscles. In addition, the prevalence of intestinal parasites in various bird species was assessed at a zoological garden in Egypt. From the studied birds, 72 fecal samples were collected at random. It was found that 63.9% of the samples had at least one intestinal parasite. The next most common test was for helminths, with 27.8% testing positive, followed by protozoa with 36.1%. Parasite species identified included *Ascaridia* spp., *Heterakis* spp., *Capillaria* spp., *Contracaecum* spp., Strongyloide savium, Strongyloides pavonis larvae, *Eimeria* spp., and *Cryptosporidium* spp. Strongyloides pavonis, *Contracaecum* species, and E. mutica are among those reported for the first time in Egypt (El-Shahawy & Abou Elenien 2015). Wild passerine birds in Britain and Ireland were studied for parasite prevalence, abundance, and infection intensity. Across 13 sites, 755 feces samples were collected from 18 bird families. Corvids and finches had the highest parasite prevalence and intensities. In terms of genera, *Syngamus* (33%) and *Isospora* (32%) constituted the most prevalent species observed. Avian families and seasons vary in parasite prevalence and abundance, while regions and families differ in infection intensity. (Parsa *et al.* 2023).

In Federal University of Agriculture Zoo Park, there are five genera of gastrointestinal parasites, namely *Ascaridia, Ascaris, Capillaria, Strongyloides,* and *Rallietina.* At different weeks of collection, three different parasites infected the Ostrich, Owl, and Mallard ducks. In both the ostrich and the owl, mixed infections were observed. Birds surveyed had a high prevalence of Capillaria spp, which was found in 57.1% of cases. In order to reduce infection levels among birds at Zoo Park, it may be necessary to improve the hygiene of birds' cages (Sam-Wobo *et al*. 2017). A total of 276 poultry samples were collected from different villages in Aswan, Upper Egypt where there was prevalence of intestinal helminths in chickens and pigeons. Baladi chickens had helminth infections on average of 55.79%, and 59.09% had mixed infections with four cestodes and three nematodes. Cestodes belonging to the *Raillietina tetragona, Raillietina echinobothrida, Cotugnia digonopora, and Raillietina cesticillus* families were most prevalent in baladi chickens. There were three dominant nematodes: *Heterakis gallinarum, Ascaridia galli,* and *Subulura brumpti. Ascaridia columbae* and *Raillietina cesticillus* were common cestodes and nematodes in pigeons 52.5% infested with Cestodes and Nematodes, respectively. Intestinal helminth occurrence was higher in adult birds than in young birds. Furthermore, summer infection rates were higher and winter infection rates were lower (El-Dakhly *et al*. 2019).

Gurung & Subedi (2018) study the prevalence and types of gastrointestinal parasites in pigeons at three temples in the Pokhara valley. There was an overall prevalence rate of parasitic infection of 69.16%, with helminths being more prevalent than protozoan parasites. Among the temples, Bhadrakali had the highest prevalence of gastrointestinal parasites, followed by Tal Barahi and Bindhyabasini. As far as gastrointestinal parasites are concerned, no activities were found in the study regarding the care of pigeons. Consequently, pigeons in the Pokhara valley temples are more likely to acquire gastrointestinal parasites. The study found that 40% of all poultry examined were infected with several species of parasites, with the highest prevalence rate observed in *Heterakis gallinarum*. Free-range chickens had a higher prevalence of infection than poultry chickens from farms due to their feeding habits and unhygienic environment. There were statistically significant differences in helminth parasite prevalence between free-range and poultry chickens, with free-range chickens mostly infected with one or more helminth parasites (Mujahid 2017). In Kathmandu valley, two temples were investigated for the prevalence of gastrointestinal parasites in feral pigeons. The prevalence of positive fecal samples was 90.83% out of 120 samples examined. A total of six parasites were identified, including *Eimeria* sp. (43.34%) and five helminth genera, with *Capillaria* sp. dominating (51.67%). The prevalence of helminths (83.34%) was higher than that of protozoan parasites (43.34%), and mixed infections were more common (50.84%) than single infections (40%). It was found that there were no significant differences in infection rates between the two study areas or between single and mixed infections. Among multiple infections, double infections were the most common. In this study, it was demonstrated that feral pigeons have a high susceptibility to gastrointestinal parasites. In order to control parasitic infection and reduce feral temple pigeon health hazards, sustainable measures must be implemented (Jha 2017).

A total of 155 fresh fecal samples were collected and examined in pigeons in Ratnanagar Municipality, Chitwan, Nepal. The samples revealed 87.1% prevalence, with 16 parasite species, including 8 protozoa and 8 helminths. A higher prevalence rate and parasitic richness were found in temple pigeons than in household pigeons. It was found in both types of populations that mixed infections belonging to up to four species were present. In order to control parasitic infections in pigeons, the study recommends proper management and deworming practices (Adhikari *et al*. 2022). Similar research examined 77 samples of pigeon feces and found that 41 (53.24%) were positive for intestinal protozoan and helminth parasites. The most prevalent parasite was *Ascaridia columbae* (27.27%), followed by *Eimeria* spp. (22.08%), *Capillaria* spp. (11.68%), and *Raillietina* spp. (5.19%). Among the positive samples, 24 (58.54%) were positive for helminth parasites and 17 (41.46%) were positive for protozoan parasites. The prevalence of multiple infections was found in 19 (46.34%) samples, while it was found in 22 (53.66%) single samples (Sukupayo 2018).

In Tansen, Palpa, (Resmi 2021) conducted a survey on gastrointestinal parasite prevalence in barn swallows. There were 120 fecal samples collected randomly, of which 105 (87.5%) tested positive for parasitic infection. The presence of six different gastrointestinal parasites was determined, consisting of one type of protozoan (*Isopora* sp.) and five types of helminths (*Ascaridia* sp., *Echinostoma* sp., *Heterakis* sp., *Strongyle* sp. and *Trichostrongylus* sp.). Helminths were more prevalent (93.33%) than protozoa (35.24%). There was a high prevalence of GI parasites in the Bus Park and Batase Dada areas (95%). There was only one heavy infection with *Ascaridia* spp. in one sample, while six and seven samples with *Echinostoma* spp. had moderate infections. According to the study, barn swallows in Tansen, Palpa have a high risk of contracting gastrointestinal parasites. The first intermediate host must be identified and controlled with different pesticides to minimize GI infestations in barn swallows.

CHAPTER 3: MATERIALS AND METHODS

3.1. Study Area

A study was conducted from June to September, 2019 in the lowlands of Nepal, specifically in Lumbini and Koshi appu, where the nests are located. Lumbini is located in the central-west lowlands of Nepal. The climate in this region is hot in summer with an average of 38^{O}C while in winter it is about 19^{O}C. Lumbini has flat wide cultivated land in the western and southern areas, while a Churia hill with a dense forest of *Shorea robusta*, Dalbergia sissoo is situated in the northern parts. This area is home to Jagdispur wetland, one of Nepal's Ramsar sites. Koshi has been a stop-over site for migratory birds. During summer, it is hot, while during winter, it is neither too warm nor too cold. There are different habitats in this area, including wetlands, rivers, cultivated lands, dense forests of *Shorea robusta*, Dalbergia sisso, making it ideal for birds to spend the winter here.

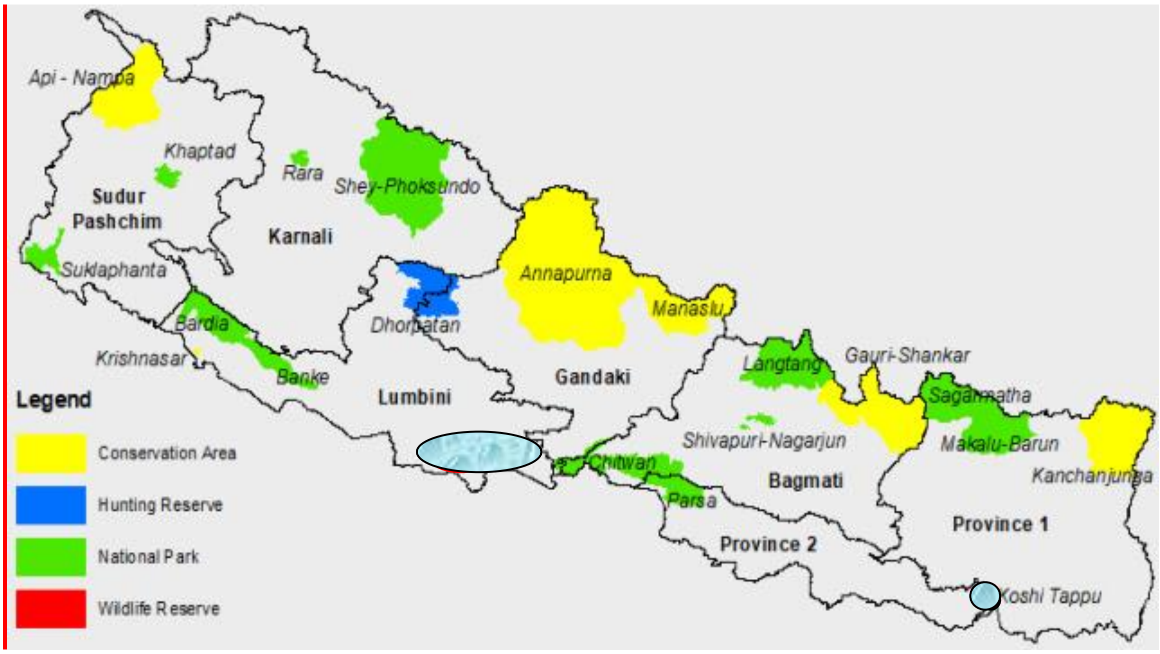

Figure 1: Map of study area: Lumbini and Koshi Tappu

3.2. Collection, Preservation and transportation of fecal samples

Fresh fecal samples were taken by the purposive sampling method from leaves and transparent green plastic sheets placed below the nesting and roosting sites of Indian spotted eagles. Samples were collected during the breeding period when they are expected to remain in their nests. Guano was collected just below the nest and near the nesting tree where the male is expected to rest. The sample collection was done mainly during chick rearing. In order to find food for their eaglets, the parents are supposed to leave their nest. The actions created least disturbance to the breeding pair. The chick rearing period typically takes place between July and September.

3.2.1. Collection by using plastic sheets

First the roosting and nesting trees were identified by direct observation and clean transparent green plastic sheets were placed in the early morning below those trees, so that when eagles defecate it may fall in those plastic sheets and the fecal samples were collected after regular observation by scrapping with sterile bamboo toothpicks from those plastic sheets.

3.2.2. Collection from leaves

The nesting sites of Eagles were high up in the trees, which meant when they defecate, their matter fell on the leaves of those plants or other plants below. Those leaves were scraped to collect feces.

3.2.3. Preservation and transportation

Collected fecal sample was immediately preserved in sterile leak proof vial containing 2.5% potassium dichromate and transported safely to the laboratory of Central Department of Zoology (CDZ), Kirtipur, Kathmandu, Nepal.

3.3. Laboratory examination and identification

Fecal samples were then processed and subjected for microscopic examination. Identification of ova/oocysts/cysts and larvae of different parasites were done according to the morphology and quantitative estimation by using concentration methods (floatation and sedimentation) and Stoll's count technique to determine mix infection and intensity of parasites(Soulseby 1982; Zajac and Conboy 2012).

3.3.1 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 faeces. Therefore, floatation and sedimentation techniques were used for concentration (Soulseby 1982; Zajac and Conboy 2012).

3.3.2 Floatation technique

This technique is based on the principle that lighter eggs/cysts of helminthes and protozoans float on mediums having a higher density. Fecal samples were mixed with a small quantity of water and filtered with tea strainers. The filtrate solution was poured into a centrifuge tube of 15 ml and centrifuged at 1000rpm for five minutes. The tube's water was replaced with a super saturated Nacl solution and centrifuged until the solution was removed. After centrifuged, a more saturated Nacl solution was added to develop a convex meniscus at the top of the tube. One drop of Methylene blue was also added. The eggs and cysts float to the top and were collected by placing a cover slip on the surface of the meniscus at the top of the tube. There was a cover slip placed for a five-minute period. It was then removed from the tube, arranged on a glass slide and microscoped at 10X and 40X. Photographs of eggs and cysts of parasites were taken and identified as nematode and cestode eggs(Soulseby 1982; Zajac and Conboy 2012).

3.3.3 Sedimentation techniques

This technique is based on the principle that parasitic eggs having high density than the suspended medium settle and concentrate at the bottom of the medium. The supernatant solution of floatation was removed and the sediment at the bottom was taken out with a long pipette. Iodine wet mount was prepared for each sample by mixing 1-2 drops of the sediment with Lugol's iodine solution on a glass slide. It was observed under examined under 10X and 40 X microscope magnifications. It is usually used for trematode eggs and not suitable for protozoan cysts.

3.4. Identification off eggs and larvae of parasites

Slides prepared using various techniques were examined under a microscope at 10X and 40X respectively. Size of eggs/oocysts/cysts was measured through stage and ocular micrometer. Identification was done using books Soulsby (1982), Zajac and Conboy (2012) and other published and unpublished articles and internet sources based on morphological characters (shape and size).

3.5 Determination of Intensity of parasites

Stoll's method of egg counting was used for determining the intensity of parasites. Fecal samples were mixed with appropriate amount of N/10 NaoH in a thick glass tube and were vigorously shaken to make a uniform emulsion. Exactly 0.15ml of the emulsion was taken by a measuring pipette and placed in a large slide 3"x2" size and covering with 22/40mm cover slip. The number of eggs per gram of feces was obtained by multiplying the count of two such preparations by 100 and considering the consistency of fecal sample correction factors (c.f.) employed. (Stoll and Hausheer 1926).

3.6. Data Analysis

MS Excel 2010 was used to enter the data on parasite presence and absence, from which the prevalence of the parasites was determined. Data were statistically analyzed using chi-square and P value performed by R-software (r-3.4.1).

CHAPTER 4. RESULTS

Various infectious diseases negatively affect the health and well-being of birds. Among them, parasitic diseases are overlooked but emerging health problems for birds of prey. The various parasites were also found in Indian spotted eagles found in Lumbini and Koshi Tappu of Nepal. The overall frequency of gastrointestinal parasites was 32.5%

4.1. Species wise prevalence of parasites

A total of 40 samples, one sample was positive for protozoa, and 7 samples showed positive results with helminths parasites. A protozoan parasite, *Isospora* sp, was the only one identified among the identified parasites. Among helminths, *Neodiplostomum* sp. had the highest prevalence followed by *Nematostrigea* sp. *Strigea* species and *Strongyle* sp. were observed (Table 1).

Table 1. Species wise prevalence of parasites

Class	Parasites	Prevalence
Protozoa	*Isospora* sp.	7.69%
Trematodes	*Neodiplostomum* sp.	23.07%
	Nematostrigea sp.	7.69%
	Strigea sp.	7.69%
Nematodes	Strongyle sp.	15.38%

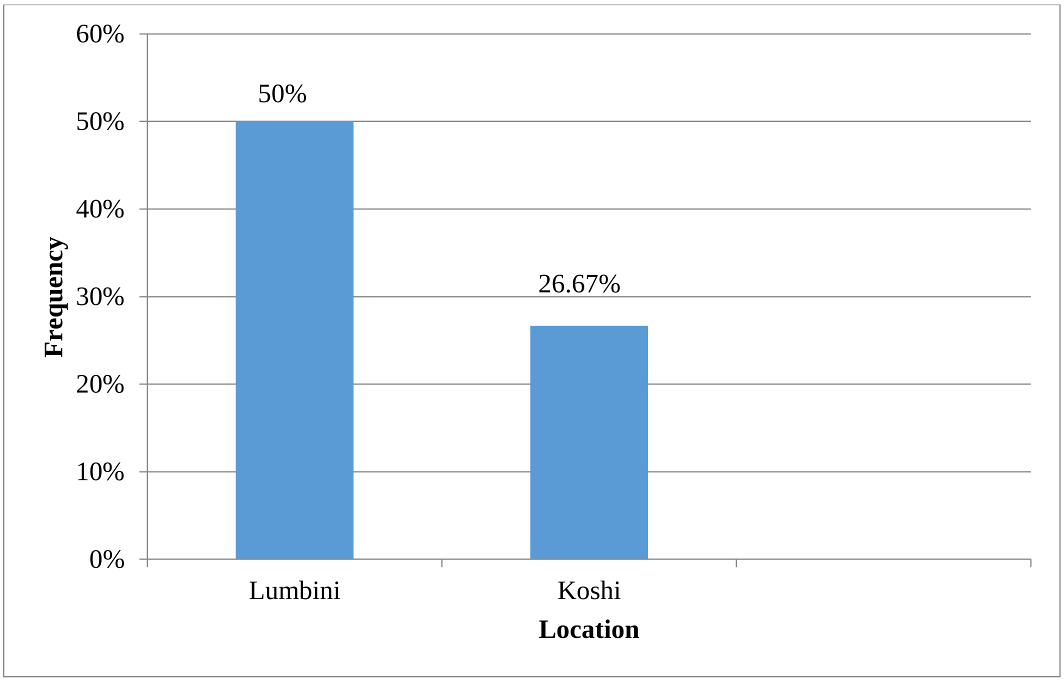

Figure 1. Frequency of GI parasites in different locations.

Indian spotted eagle feces have been collected from two exceptional sites, Lumbini and Koshi. A higher number of parasitic infections were found in feces collected from Lumbini compared to Koshi (Fig. 2).

In two lowlands of Nepal, parasite prevalence was comparable, showing that most parasites such as *Neodiplostomum* sp. *Nematostrigea* sp., *Strigea* sp. *Strongyle* sp. is common in both places but only protozoa i.e. *Isospora* sp were found in Lumbini. Statistical comparison of parasites at two different sites showed the prevalence of parasites was unchanged between the two locations

Indian spotted eagles were found to be infected with a single parasitic infection or multiple parasitic infections. Most of them were infected. By single parasite species followed by double and triple infections. Statistically, most of the single infections were caused by *Neodiplostomum* sp. Indian spotted eagles were infected with either only one parasitic infection or multiple. A maximum of them had been contaminated via single parasite species observed by double and triple infections. Statistically, among the single infections, most of them are infected with the *Neodiplostomum* sp. There was no significant difference in the frequency of parasitic infections among the two groups. (X^2= 0.36, df= 2, p>0.05).

Table 2. Frequency and association of GI parasites in different locations

Parasites	**Lumbini (n=20)**	**Koshi (n=20)**	**X^2 value**	**P value**
Isospora sp.	12.5%	0	0	1
Neodiplostomum sp.	25%	20%	5.92	1
Nematostrigea sp.	0	20%	0.34	0.55
Strigea sp.	12.5%	0	0	1
Strongyle sp.	12.5%	20%	0	1

A variety of specific mixed infections were present among the multiple infections. In double infection maximum of 3 distinct types of parasitic combinations of mixed infection were observed.

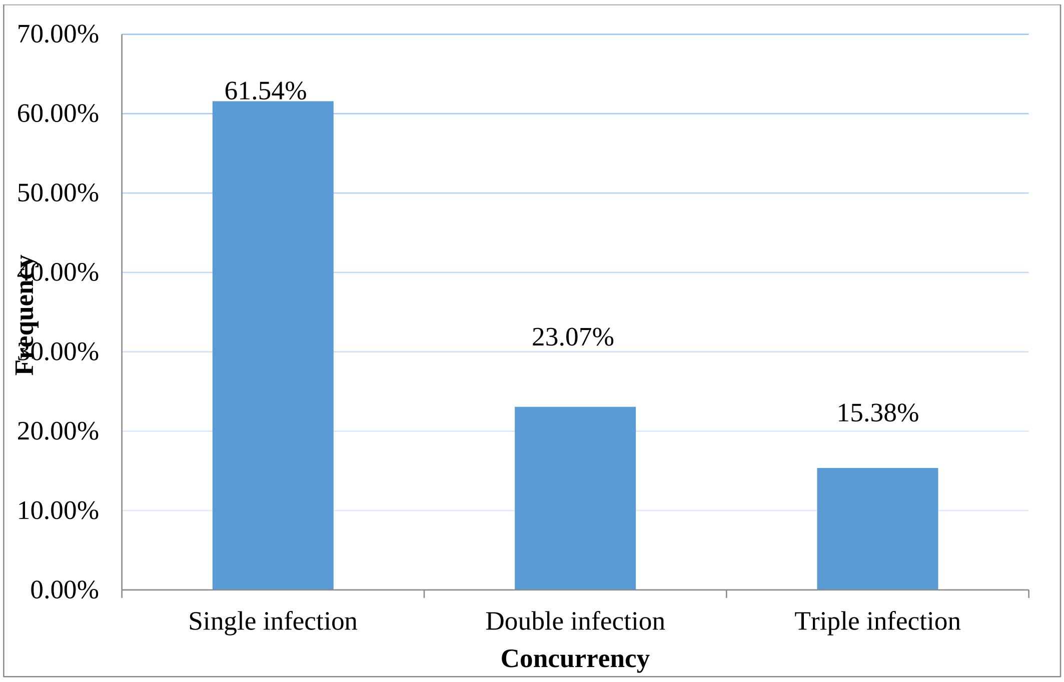

Figure 2. Concurrency of GI parasites

Table 3. Parasitic specific mix infection

Mixed infection	**Combination**	**Total**
Single infection		38.46%
Double infection	*Neodiplostomum* sp. +*Strongyle* sp.	7.69%
	Nematostrigea sp. + *Strigea* sp.	7.69%
	Isospora sp. + *Strongyle* sp.	7.69%
Triple infection	*Neodiplostomum* sp. + *Strigea* sp. + *Strongyle* sp.	7.69%
	*Nematostrigea*sp. + *Strigea* sp. + *Strongyle* sp.	7.69%

Triple infection observed two different combinations. *Strongyle* sp made a maximum of 2 different combinations of mixed infection in double infection and two different combinations in triple infection followed by *Striga* sp which made one combination in two infections and two combinations in triple infections (Table 3). A comparison study of parasite concurrency in various locations showed a variety of variations. Lumbini got the highest single, multiple, and triple infections compared to Koshi. Triple infections were equal at both sites. In comparison, the Lumbini location showed high concurrency and Koshi showed the least concurrency of parasites (Figure 3).

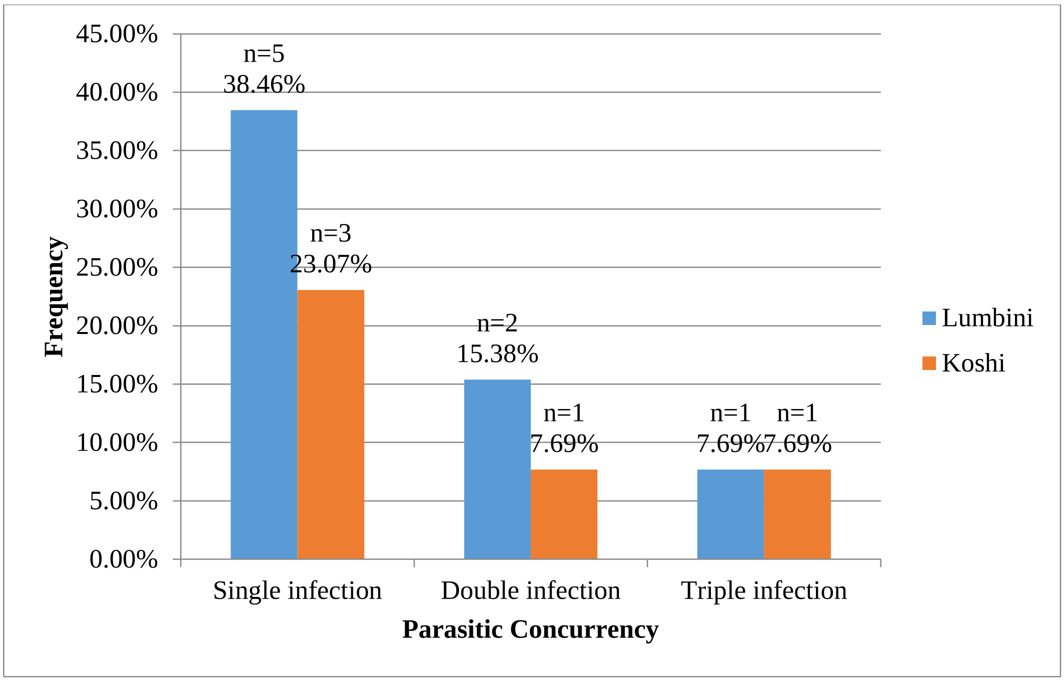

Figure 3. Concurrency of GI parasites in different locations

4.2 Intensity of parasites in Indian Spotted Eagle

In this study, heavy infection was considered as those samples with six or more cysts/egg of larvae were observed per field. Out of 8 single parasitic infections, the intensity of *Neodiplostomum* sp was found to be the most infective gastro-intestinal parasites followed by *Strongyle* type, *Nematostrigea* sp., *Strigea* sp and *Isospora* sp.

Table 4. Intensity of Parasites in ISE

S.N.	Class	Parasites	Intensity			
			Light	**Mild**	**Moderate**	**Heavy**
1.	Protozoa	*Isospora* sp.	1	-	-	-
2.	Trematodes	*Neodiplostomum* sp.	-	-	1	2
3.		*Nematostrigea* sp.	-	-	1	-
4.		*Strigea* sp.	-	-	1	-
5.	Nematodes	*Strongyle* type	-	2	-	-

Note:
Light infection=<2 eggs/oocysts/larva per field
Mild infection=3-4 eggs/oocyst/larva per field
Moderate infection=5-6 eggs/oocysts/larva per field
Heavy infection=>6 eggs/oocyst/larva per field

CHAPTER 5. DISCUSSION

This study indicates that Indian Spotted Eagles in Nepal's lowlands are relatively low of gastrointestinal parasites. There was a positive result for one or more of the gastrointestinal parasites in only 13 of the 40 fecal samples tested. The findings of the current study are consistent with those of previous studies that reported low prevalence rates of gastrointestinal parasites in birds of prey, including wild birds (Badparva *et al.* 2015, Parsa *et al*. 2023).In contrast,(Jha 2017, Sukupayo 2018, Resmi 2021) and (Adhikari *et al*. 2022) documented that the prevalence of gastrointestinal parasite is more likely to occur than in the present study. However, several factors may have contributed to the variation in the prevalence of gastrointestinal parasites in present study. These factors include the small sample size and the specific geographical location of the study site (Parsa *et al*. 2023). The parasitic infection rate may be higher in other bird populations in different regions.Indian Spotted Eagles and other birds of prey should be monitored for parasite infections even with the low prevalence of gastrointestinal parasites in this study, as parasitic infections can have significant impacts on health and fitness. The prevalence and intensity of parasitic infections in these birds can provide conservationists with useful information for protecting their populations and ensuring their long-term survival.

An Indian Spotted Eagle population was found to be infected with several gastrointestinal parasites during the study. Over the course of analyzing 40 samples, five different species were identified. It was only *Isospora* sp. that was observed as a protozoan, while the remaining four were helminths. It is reported here that the overall infection rate of protozoan parasites was 7.69 %. These findings are similar to previous studies that reported helminth infestations to be 8.01% and protozoan infections to be 11.1% in Chattogram, Bangladesh (Bayzid *et al*. 2023). In terms of prevalence, this finding is a relatively lower prevalence than reported previously. This was 24 (58.54%) positive for helminth parasites and 17 (41.46%) for protozoan parasites (Sukupayo 2018). It is possible for endoparasitessuch as protozoa and worms to invade various organs in their hosts. These organisms pose significant health problems for companion birds and must be considered when making a differential diagnosis in newly acquired birds and large collections of aviaries (Ombugadu 2018). A high prevalence and intensity of infection have been found to accompany gastrointestinal parasitism in selected parrots, according to (Ombugadu 2018) and this type of parasitism occurs most often in caged and aviary birds. Endoparasites can cause major problems for captive birds, particularly in poultry farming. Birds with these parasitic diseases have lower productivity, slower growth, reduced eggs production, and poor health (Philips 2000). Birds affected by endoparasitism experience loss of appetite, vocal changes, ruffled plumage, breathing difficulties, weight loss, bloody diarrhea, and even death as a result of the parasite (Philips 2000).There has been extensive research conducted on captive birds' endoparasites to mitigate these issues.

Avian species are commonly affected by Helminths. There is increasing recognition that helminth parasites pose a major threat to health and productivity (Uhuo *et al.* 2013). The helminth parasites that affect birds worldwide include nematodes, cestodes, and trematodes (Naphade 2013). These infections can harm infected birds' growth, development, and immune system, especially young ones(Dauda 2016). Seven samples tested positive for *Neodiplostomum* species, which is particularly prevalent among helminth parasites. *Neodiplostomum* sp. have been reported to be a common parasite of birds of prey in previous studies (Philips 2000, Mujahid 2017, Ombugadu 2018). In terms of helminth, a variety of factors, such as climate, environment, and nutrition, can influence the prevalence and intensity of helminth infections in poultry (Ola-Fadunsin *et al*. 2019). As a result of these factors, parasite population dynamics may change, resulting in significant changes in infection prevalence and intensity (Jegede *et al*. 2016).

In this study, gastrointestinal parasites of Indian spotted eagleswere investigated in Lumbini and Koshi Tappu of Nepal. There was a higher prevalence of parasitic infections in Lumbini than in Koshi, according to the study. It may be because of the type of habitat the birds live in or the availability of food, which is affected by human activities (Aponte *et al*. 2014). Most helminth parasites were common in both locations, while protozoan parasites were only found in Lumbini. In this study, it was found that Indian spotted eagles were commonly infected with parasites, which highlights the importance of understanding the potential impact of these infections on their health and population dynamics. Generally, infected birds have been infected with a single parasite species, *Neodiplostomum sp*. It is noteworthy that the concurrency of parasitic infections was not significantly different. There are no noticeable differences in infection rates among eagles exposed simultaneously to multiple parasites. There is a need to continue monitoring

Indian spotted eagles for parasitic infections, as these findings indicate. Further research is needed to understand the effects of parasitic infections on the health and well-being of this species.

This study found that Indian spotted eagles are often infected with multiple parasitic species, with diverse combinations of parasites. Among the double and triple mixed infections, Strongyle species were the most common, followed by Striga species. Lumbini had higher incidences of single, double, and triple infections than Koshi, but the combinations of mixed infections varied between the two locations. Lumbini showed a high parasite concurrency, while Koshi showed a low one. The results suggest that parasitic infections may differ based on the location. It is imperative to consider the intensity of parasitic infections as it provides information about the severity of the infection and its potential impact on the host. The term "heavy infection" was used in the current study to describe fields with six or more cysts/eggs per field. Based on the intensity of the eight intestinal parasites identified in Indian Spotted Eagles, *Neodiplostomum* species was found to be the most highly infectious, followed by *Strongyle* type, *Nematostrigea* sp., *Strigea* sp., and *Isospora* species. To determine the ecological and conservation implications of these findings, further research will be necessary to determine if *Neodiplostomum* species play a more significant role in Indian Spotted Eagle health.

CHAPTER 6. CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

The Indian Spotted Eagle is infected with parasites, including helminths and protozoa, In Lumbini and Koshi Tappu of Nepal. The overall frequency of gastrointestinal parasites in Indian Spotted Eagles had 32.5%, and *Neodiplostomum* sp. is the most prevalent parasite. The prevalence of parasitic infections was higher in Lumbini than in Koshi. The prevalence of parasites in the two locations did not differ significantly. Additionally, Indian Spotted Eagles displayed a variety of infections, including combinations of specific infections and mixed infections. The highest level of infection was found in *Neodiplostomum*sp, followed by *Strongyle*sp. and *Nematostrigea* sp. followed by *Strigea* sp. and *Isospora* sp.

6.2 Recommendation

On the basis of conclusion, following recommendations have been proposed.

- Further identification on species level of parasites could be done.
- Further study by collecting adequate amount of sample is suggested.

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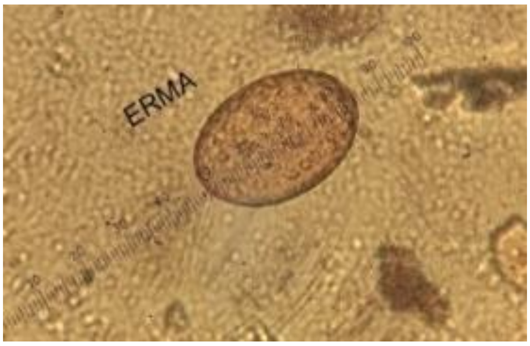
APPENDIX

Egg and larvae of parasites in Indian Spotted Eagle under 10X*40X electronic microscpe.

Photo1: Strogyloides sp. (384 µm)

Photo 2: *Nematostrigea* sp.(100.8µm)



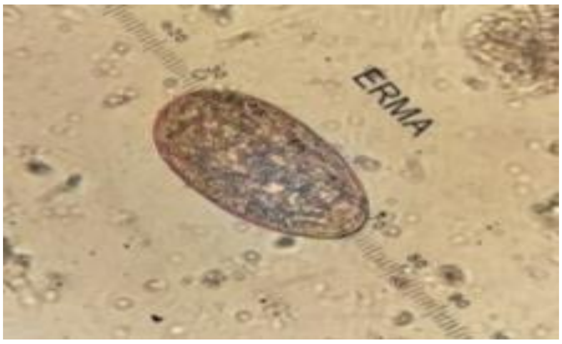


Photo 3: *Strigea* sp. (86.4µm)

Photo 4: *Neodiplostomum* sp.(98.4µm)

Photo 5: *Isospora* sp. (24µm)