GASTROINTESTINAL PARASITES OF QUAIL IN SIDDHARTHANAGAR, RUPANDEHI, NEPAL

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Submitted to

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Institute of Science and Technology

Tribhuvan University,

Kritipur, Kathmandu

Nepal

March 2023

DECLARATION

I hereby declare that the work presented in this thesis entitled **"Gastrointestinal parasites of quail in Siddharthanagar, Rupandehi, 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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LETTER OF APPROVAL

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Niru Kanauje entitled "**Gastrointestinal parasites of quail in Siddharthanagar, Rupandehi, Nepal"** has been accepted as a partial fulfillment of the requirements for Master's Degree of Science in Zoology with special paper Parasitology.

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TABLE OF CONTENTS

LIST OF TABLES

Table No. **Title of the tables** **Page No.**

LISTS OF FIGURES

Figure no. **Title of Figures** **Page no.**

LIST OF PHOTO PLATES

LISTS OF ABBREVIATIONS

Abbreviation form	Details of abbreviation
CDZ	Central Department of Zoology
et al.	ET alia (and other)
Fig.	Figure
GDP	Gross Domestic Product
GI	Gastrointestinal
i.e.	That is.
no.	Number
P value	Probability value
Rpm	Rotation per minute
Sp.	Species
χ^2-	Chi square
µm	Micrometer

ABSTRACT

Quails are primarily reared for meat and eggs which plays a key role in enhancing the economic status of local people. The study aimed to determine the prevalence of gastrointestinal parasites of quail in Siddharthanagar, Rupandehi, Nepal. A total of 150 fecal samples of quail including 65 from young and 85 from adults were collected from December 2021 to May 2022. The examination of fecal samples was done by direct wet mount method and concentration methods (flotation and sedimentation technique) in the laboratory of Central Department of Zoology, Kritipur. The study found that 72.67% of the fecal samples were found positive for gastrointestinal parasites. Among identified GI parasites, *Eimeria* sp. (29.33%) was found to be the most prevalent parasite followed by *Ascaridia* sp. (21.33%), *Heterakis* sp. (16%), *Capillaria* sp. (12%), *Strongyloides* sp. (7.3%) and *Raillietina* sp. (4.6%). The prevalence rate of gastrointestinal parasites was found in young (78.46%) and in adults (68.23%) with no significant difference. Moreover, the study found that there was not significantly different between seasons, with an infection rate of 78.67% in winter and 66.67% in summer. Single parasitic infections were more common than double infection. The results indicate that quails are highly susceptible to gastrointestinal parasites and need to undertake preventive measures for controlling the risk of parasitosis in quail.

Keywords: Quail, Gastrointestinal parasites, Prevalence

1. INTRODUCTION

1.1. Background

Poultry production is generally referred to as an industry with a connection to agriculture. Even though chicken is the most common type of poultry, the phrase also includes other birds including turkey, quails, duck, guinea fowl, and geese. Nepal is an agricultural country, so agriculture accounts for a sizable portion of national GDP (29%). Poultry farming is one of the fastest growing agricultural sectors in Nepal, accounting for approximately 4% of national GDP. The poultry sector includes both backyard poultry and commercial poultry, with backyard poultry accounting for approximately 45 percent of total poultry production and commercial poultry accounting for the remaining 55 percent (Nirmal & Pokharel 2017). Boosting the poultry production as a commercial enterprise in Nepal began three decades ago and has expanded dramatically over the last six decades, generating income in both urban and rural areas (Shreshtha 2018).

Quail is a small, stocky bird with short legs and a variety of plumage colors. It is a member of the Phasianidae family, which also includes pheasants and partridges. The wild variety of common quail (*Coturnix coturnix*) measures 16-18 cm and weighs 70-135g (Jubril et al. 2021). Quails are found in 45 different species around the world. Among 45 species, Japanese quail (*Coturnix coturnix japonica*) and Bobwhites quail (*Colinus virginianus)* were the two most important species that considered domestic birds since 14th century (Arya et al. 2018). The Japanese quail is a migratory bird belonging to order Galliformes, family Phasianidae, *Coturnix* genus and species *japonica* is the most efficient and economic value as a commercially farmable species raised primarily for its meat and eggs (Priti & Satish 2014). They were originated in Asia, North Africa, and Europe. Because of the rise in the consumption of exotic eggs and meats, they were viewed as a viable alternative to the production of chicken (Elmorsy et al. 2020).

Nepal has two breeds: Pharaoh (with a rusty brown underbelly and a natural brown color on the head and upper body) and British Range (Red-brown chocolate all over). The male has a red patched below the neck, whereas the female has a black or grey spot. Male body weight ranges from 100-140gm, while female body weight ranges from 120-150gm. The female lays 280 eggs per year (Nirmal & Pokharel 2017).

The need for more animal protein sources is driven by the persistent growth of the human population due to which quail production is one of the most rapidly growing poultry industries in developing countries. Meat and egg production are the most common reasons for raising these birds (Mohammed & Ejiofor 2015). Quail production is more profitable due to low initial investment, high laying rate, low feed, good growth, minimal space requirements, low mortality percentage and yields faster returns with a greater cost-benefit ratio (Redoy et al. 2017). Additionally, it also serving as a valuable resource for behavioral and biological studies; however quails are vulnerable to many helminths and protozoan diseases (Islam et al. 2020).

Quails are found to be more resistant to many poultry diseases than chicken, but due to poor management practices, the stress of intensive rearing, poor hygiene, and other factors, these species have become susceptible to a wide range of potentially harmful organisms, including bacteria, viruses, and parasites (Monte et al. 2018).Many factors, including bird age, infective dose, and poor management, increase the incidence of both helminthic and protozoan infection in quail (D'souza et al. 2022).

1.2. Parasites in Quail

Parasites are living organisms that receive nourishment and shelter from other organisms where they live. Endo-parasites known as intestinal parasites adhere to the intestinal wall and feed on the food in the lumen and intestinal wall, causing harm to the host in the process (Shrestha et al. 2020). The most common and destructive parasites impacting quail productivity, nevertheless, are gastrointestinal parasites causing significant losses in many poultry farms. The gastrointestinal tract plays a significant role in digestion and absorption of foods; so, it may lead to improper food absorption and slow growth performance as well as disruption of production if any changes occur in intestinal health and digestion. The gastrointestinal tract of the quail is invaded by some parasites such as protozoans, nematodes, cestodes, acanthocephalans, and trematodes (Soulsby 1982). These parasites cause diarrhea, lack of appetite, ruffled feathers, impaired intestinal absorption, weakness, nervous disorder, anemia, reduce weight gain, reduction in growth rate, drop in egg production, high mortality, and ultimately substantial economic loss (Islam et al. 2020).

Quails are vulnerable to a variety of infectious diseases. Almost every common chicken disease has been reported in quails (El-Ghany 2019). There are some viral and bacterial disease like bird flu, Newcastle disease, Avian pox, chronic respiratory disease etc. that causes high mortality and morbidity (Khadka 2019). Bacteria, viruses, parasites, and even non-infectious factors such as poor management and dietary deficiencies can all cause intestinal problems in quail. Because backyard flocks are a reservoir for several diseases, their low biosecurity may be a problem for commercial poultry flocks (Derksen et al. 2018). Various endoparasites especially protozoan and helminths cause directly and indirectly heavy loss in poultry farming.

There are numerous helminth parasite species that affect quails. Helminths are classified according to their location. For example, worms that are found in the caecum of the large intestine are known as cecal worms (*Heterakis* sp.) causes the disease Histomoniasis in poultry (Cupo & Beckstead 2019). *Heterakis gallinarum* infection usually do not manifest any clinical symptoms, but they are a significant host for *Histomonas meleagridis* (McDougald 2005), eye worms (*Oxyspiruramansoni*) that are located in the eye, and gap worms (*Syngamus trachea*) which are found in the trachea (Gauthier & Ludlow 2013). *Ascaridia* sp. is found in the small intestine that cause loss of body weight, intestinal hemorrhage, and an increase in mortality as a result of minor intestine blockage (Islam et al. 2020). *Ascaridia galli* research in wild and migratory birds is critical for determining the risk that this parasite will be transmitted to local bird populations during migration (Faizullah et al. 2021).

Another nematode, *Capillaria* sp. are long, and slender that may have a direct or indirect life cycle and are not host-specific. Most commonly, they reside in the small intestinal mucosa, although some also inhabit the esophagus, cecum, and crop. *Capillaria* sp. produces inflammatory lesions as well as thickened crop and esophagus (Permin et al. 1999). Cestodes are segmented, flat tapeworms. *Choanotaenia* sp. infects the posterior part of the small intestine of birds and *Raillietina* sp. can involve the posterior part of the small intestine of the birds (Eslami et al. 2009; Mamashly et al. 2010). Trematodes are dorsoventrally flattened flukes with an oral sucker and, in some cases, an acetabulum. *Echinostoma* trematode of small intestine of birds and are found on the area where there are suitable conditions for growth of intermediate host that are molluscans (Permin & Hansen 1998).

Acanthocephalans are thorny-headed worms due to the spiny proboscis that attaches to the intestine of the host, but these infections are rare and are sometimes considered accidental infections. Trematodes and acanthocephalans are also poorly represented in quail (Kellogg & Calpin 1971).

Coccidiosis is one of the most pathogenic protozoan diseases caused by species *Eimeria* resulting in great economic losses worldwide (AlZarkoushi & AlZubaidi 2021). Coccidia parasites are extremely species-specific, and once the coccidia have completed their life cycle, acquired immunity can be attained. However, since birds may both carry the illness and become carriers after they become infected, the likelihood of coccidiosis spreading is increased (Williams 1998). The disease is divided into two categories based on the organs it affects: intestinal coccidiosis, which affects the small intestine, and cecal coccidiosis, which affects the large intestine (caeca). Several *Eimeria* species have been described from various quail species (genus *Coturnix*) that are not host specific (Teixeira et al., 2004). *Eimeria coturnicis* was described from common grey quail, *Eimeria tahamensis* was reported from Arabian quail (*Coturnix delegorguei arabica*), and *Eimeria uzura, Eimeria bateri, and Eimeria tsunodai* (Berto et al., 2013) was described from Japanese quail (*Coturnix coturnix japonica*). Dysentery, enteritis, emaciation, drooping wings, poor growth, and low output are all signs of coccidiosis (Shirzad et al. 2011).Worms and protozoa such as *Eimeria* sp., *Cryptosporidium* sp., and *Trichomonas* sp. are common parasites that infect the digestive tract of quails (Hassan et al. 2020).

Parasitic disease is a problem wherever poultry are reared, whether in large commercial operations or small backyard flocks, and causes significant losses in the poultry industry (Poudel et al. 2020). A few parasites do not usually cause a problem in most cases, but large numbers can have a devastating effect on growth, egg production, and overall health (Tesfaheywet et al. 2012). Domestic fowls are more frequently infected due to poor management systems, a lack of veterinary services, and a lack of knowledge about parasites. Parasites cannot be completely eradicated, but their numbers can be controlled. To prevent such infection, farmers should be educated about the risks posed by the various gastrointestinal parasites associated with quail and poultry in general.

1.3 Objectives

1.3.1 General objective

- To determine the general prevalence of gastrointestinal parasites of quail in Siddharthanagar, Rupandehi, Nepal.

1.3.2 Specific objectives

- To determine the prevalence of various groups of gastrointestinal parasites of quail.
- To determine the prevalence of gastrointestinal parasites of quail based on age and seasonal.

1.4 Significance of the study

As the world's human population grows the demand for animal protein becomes an increasingly important nutrient component. Aside from their many uses, quails are vulnerable to many helminths and protozoan diseases due to their mixed farming system, which hampers the farming system with significant economic loss. So, by identifying parasites and bringing in management of quails by providing a hygienic environment, healthy feed, and veterinary suspension, this study may help to reduce infection in quails. The discovery of new helminths and protozoan species in Nepal will help future research on gastrointestinal parasites in quail.

2. LITERATURE REVIEW

Quails are vulnerable to a variety of infectious diseases. Many factors such as poor management systems, nutritional status, parasite ecology, and the host parasite relationship all have a significant impact on the occurrence of helminths and protozoan infection in poultry (Shrestha et al. 2020). Parasitism is one of the major problems that affect the productivity and performance of fowl (Hassan et al. 2020). Helminthes and protozoan parasitic disease were found as a major poultry disease of parasitic origin. The prevalence of various parasites in Bobwhite quails was conducted by (Alan Kocan et al. 1979) in Oklahoma identified several nematodes, cestodes and protozoa in the intestines of quail with 45% *Trichomonas* sp. being the most prevalent followed by 27% *Subulara brumpti*, 4% *Heterakis gallinarum*, 6% unidentified cestodes, 30% *Chilomastix* sp., 27% *Eimeria* sp., 25% *Trichomonas gallinarum* and 7% *Histomonas melegridis*. A survey on 40 Bobwhite and Japanese quails conducted in Iran (Shemshadi et al. 2014) found that the dominant parasite was protozoan (52.5%), with intestinal and tracheal cryptosporidiosis being the most common, and helminthic infections being less prevalent (5%) of the quails harbored *Raillietina echinobothrida* and *Raillietina cysticillus*. This similar result was found in the study by (D'souza et al. 2022) from India showed the higher prevalence of protozoan infection (29.33%) compared to helminth infection (13.2%). However, (Rosa et al. 2017) from Brazil revealed higher prevalence in helminths (56%) than protozoa (40%) in quails. Overall, these studies demonstrated that quails were susceptible to various types of parasitic infections, with varying prevalence rates in different regions.

In a study of the endoparasites in quail where 200 fecal samples from quail farms in Bangladesh were examined by direct smear methods and found an overall prevalence of 17.5% nematode eggs. The most prevalent nematode parasite was *Heterakis gallinarum* (7%) and the least prevalent was *Trichuris* sp. (1.5%) and showed a higher prevalence rate in (2.10%) winter than (1.89%) spring and (1.26%) summer (Islam et al. 2020). Similar study in Umuahia, Nigeria had reported the prevalence of 55.67% GI parasites of quails infected with helminths and 19.6% with coccidian oocysts. *Ascaridia* sp. (21.0%), *Heterakis* sp. (13.6%) and *Capillaria* sp. (11.0%) were the most frequently found nematode parasites. The prevalence of gastrointestinal nematodes was higher in adult quails (62.0%) compared to young quails (42.2%). The

prevalence of coccidian oocysts was higher in juvenile quails (34.0%) compared to adult quails (27.5%) was found in this study (Onyeabor & Uwalaka 2021). Another study of the incidence of GI parasites in 60 quails from India found a prevalence of 36.66%. They identified *Ascaridia* sp., *Capillaria* sp. and *Eimeria* sp. in fecal samples, and one cestode in the small intestine. The study also showed 53.33% of intestinal scrapings tested positive for intestinal coccidiosis, and the remaining 46.66% tested positive for cecal coccidiosis and observed that nematodes and protozoan parasites were more common in young birds (42.2%) than in adult quails (20%) (D'souza et al. 2022). These studies revealed that the prevalence of gastrointestinal parasites in Japanese quail is influenced by various factors such as age and season and indicate that GI parasites are prevalent in quails, with nematode parasites being the most common and no trematodes were found.

The prevalence and effects of coccidiosis was conducted by (Arafat and Abbas 2018) from Egypt detected 34 out of 107 (31.78%) Japanese quails were positive for *Eimeria* sp. and exhibit signs of diarrhea mixed with blood spots, intestinal lesions and sometimes cecal ballooning. A similar study conducted by (Umar et al. 2014; Anbarasi et al. 2016) showed the caecum ballooning appearance with severe serosal and mucosal congestion and its lumen contained foul smelled necrotic materials and mixed with blood. Bashtar et al. (2010) carried out the study in the prevalence of *Eimeria* sp. in 200 Japanese quails from three farms in Saudia Arabia and found 29% infected with *Eimerian* oocysts, with no infection in chicks under one month old. Young quails (7-9 weeks old) had a high infection rate of 80%, while older birds had a lower infection rate of 21.42%. Similar study on Japanese quails in Iraq was done by (Mohammad 2012) found with overall infection rate of 49.4% *Eimeria* sp. oocysts with three species i.e., *E. uzura, E. bateri and E. tsunodai* where mixed infection by three species (46.5%) was found higher than single infection (20.9%) and among them young (61.5%) had the highest prevalence over adults (31.3%). These studies showed that age plays a significant role in the prevalence of gastrointestinal parasites in Japanese quails, with young quails having a higher risk of infection. However, the prevalence and types of parasites may vary across different regions and farms.

AL Rubaie (2014) examined 180 quails from Baghdad city, Iraq. Fecal samples were taken from small and large intestines to examine the coccidian parasites. From microscopic examination, 78.33% *Eimeria* infection with four species i.e., *Eimeria*

bateri, Eimeria tsunodai, Eimeria uzura and *Eimeria fluminensis* was found. It also showed males had a high infection rate 83.01% compared to females 71.61%. Similar study was done by (Xiang et al. 2017) from USA showed 58.7% *Eimeria* sp. infection and also found a higher infection in males 60.8 percent than females. These studies showed that *Eimeria* species causing coccidiosis in quails and as sex wise, males were found having a higher risk of infection than females.

AlZarkoushi & AlZubaidi (2021) studied the prevalence of *Eimeria* sp. with different species in Japanese quails from Iraq. 330 fecal samples were collected and examined by using both the direct and flotation methods and detected 64.54% *Eimeria* sp. where two species of *Eimeria* i.e., *Eimeria bateri* and *Eimeria tsunodai* have been identified in Japanese quails. The study also showed the higher prevalence of *Eimeria* sp. in spring (75.75%) than winter (70.70%) and summer (51.51%). But a low prevalence of (29%) *Eimeria* infection was found by (Latif et al. 2016) from Pakistan. A similar study was done by (Ahmed et al. 2017) from Egypt was found 43.90 percent *Eimeria i*nfection with various species of *Eimeria bateri, Eimeria tsunodai, Eimeria uzura, Eimeria colini,* and *Eimeria bahli*. These studies showed a high prevalence of *Eimeria* species causing coccidiosis in Japanese quails, with different species being identified in Asian and African countries. Proper control measures should be considered in quail farms by implementing good hygiene and using appropriate anticoccidial drugs treatment.

The prevalence of mixed infection with multiple *Eimeria* sp. in Japanese quails was studied by (Elmorsy et al. 2020) from India revealed three different *Eimeria* species i.e., *E. bateri, E. uzura* and *E. tsunodai* (42%). Three patterns of infection were observed: single infection with *E. uzura* (42%), single infection with *E. bateri* (16%), and mixed infection by three species (42%). A similar study was conducted by (Anbarasi et al. 2016) found that 12 of 76 Japanese quails from commercial farms in India were positive for mixed infections of *Eimeria* sp. Three *Eimeria* sp. (*E. uzura, E. bateri* and *E. tsunodai*) have been identified in Japanese quails (Gesek et al. 2014) from Iran. These studies revealed that mixed infection with *Eimeria* species can lead to significant production losses including decreased weight gain and egg production, increased mortality and increased susceptibility to other diseases.

Nepal has conducted research on the gastrointestinal parasites in various birds (ducks, pigeon, and chicken, among others). However, no sufficient research has been conducted in Nepal to map the gastrointestinal parasites in quails.

Shrestha et al. (2020) studied the prevalence rate of GI parasites in ducks from Chandragiri municipality, Kathmandu, Nepal. 120 fecal samples were examined by direct smear and concentration methods and were found 81.67% positive with one or more than one gastrointestinal parasite. Among seven different species of gastrointestinal parasites, *Ascaridia* sp. (21.67%) and *Eimeria* sp. (21.67%) followed by *Capillaria* sp. 16.67%, *Hetarakis* sp. (15%), *Tetrameres* sp. (14.16%), *Strongyloides* sp. (12.50%), *Raillietina* sp. (10.83%) and found a higher prevalence of single infection (65%) as compared to mixed infection (16.67%). Similarly, Subedi et al. (2018) from Lalitpur district showed 40 percent of all the poultry examined as infectedin chicken. Among five species of gastrointestinal parasites, *Heterakis gallinarum* (22.4%) followed by *Capillaria* sp. (16%), *Ascaridia galli* (10.4%), unidentified species (4.8%) and *Raillietina tetragona* (4%). The study conducted by Sukupayo (2018) on pigeons from Bhaktapur found a higher prevalence rate of helminth parasites(58.54%) compared to protozoan parasites (41.46%) which was also observed by (Gurung & Subedi 2018) found the prevalence of gastrointestinal parasite of (55%) helminths were higher than (19.16%) protozoan parasites. Trematodes were found in some studies of (Resmi 2021) and (Paudel 2012), with *Echinostoma sp.* in barn swallows (35.23%) and (8.89%) ducks respectively being the only one reported species. Khadka (2019) found two *Eimeria* species in Kadaknath from Suddodhan rural municipality, Rupandehi (*Eimeria tenella* and *Eimeria maxima*). A similar study was carried out in Lalitpur district, Jayswal et al. (2014) reported four *Eimeria* sp. (*Eimeria acervulina, Eimeria maxima, Eimeria necatrix, Eimeria brunette*, and *Eimeria tenella*), with *Eimeria tenella* having the greatest occurrence rate.

3. MATERIALS AND METHODS

3.1 Study Area

Siddharthanagar is a terai area of Nepal which formerly still called Bhairahawa which is situated in Rupandehi District in Lumbini Province of Nepal at a geographical coordinates of 83 26' East longitudes and 27 31' North latitudes. It covers an area of 36.03 sq. km. It has a tropical climate, summer with a warm and winter with cool, dry, and humid. Many people in this area are involved in poultry farming on both small and large scales. The maximum temperature is about 45^0C, and minimum temperature is reached up to 2.4^0C.

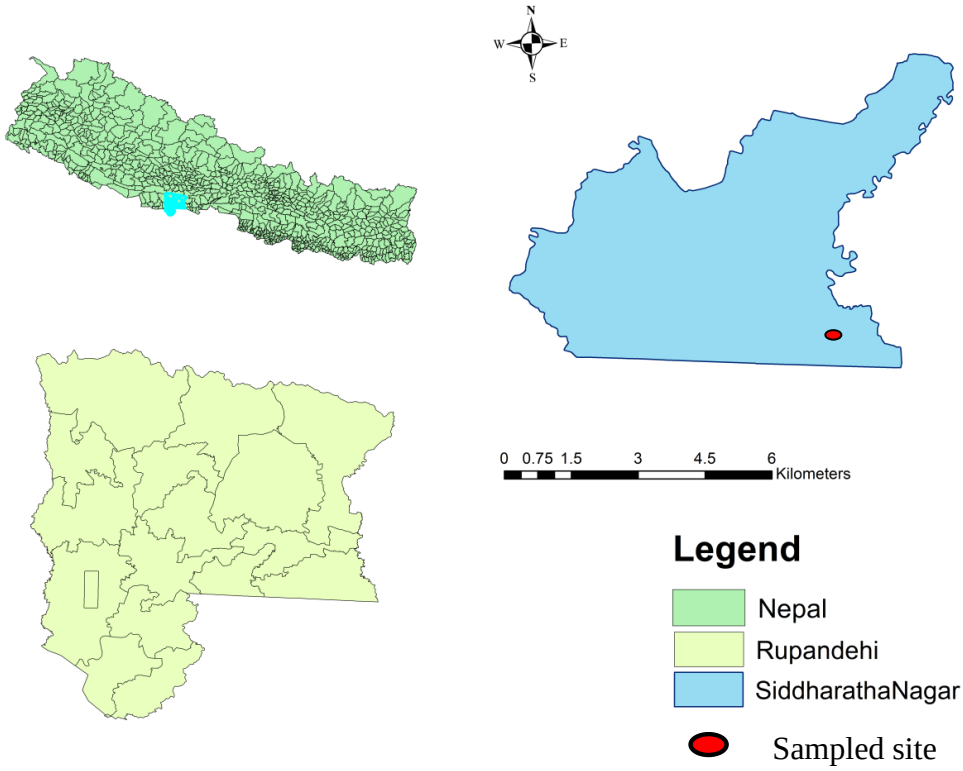

Figure 1: Map of Nepal showing Siddharthanagar, Rupandehi, Nepal

3.2 Materials

3.2.1 Materials used during field visiting:

- Vials, toothpick, and polythene bags
- Gloves and Mask
- Camera
- Pencil and paper sheet

3.2.2 Materials used during the lab work

Electric microscope, Centrifuge machine, collecting vials, Centrifuge tube, Gloves, Measuring cylinders, Beakers, Dropper, Toothpicks, Volumetric flask, Tea strainer, Glass slides, Cover slips, Stage micrometer, ocular micrometer, and camera.

3.2.3 Chemicals Requirement

2.5% $K_2Cr_2O_7$ (Potassium Dichromate), distilled water, Normal saline (0.85%), Lugol's iodine, 10% formalin, Ethyl acetate, sodium chloride, Ziehl-Neelsen (ZN) Acid fast stain, immersion oil, sodium monophosphate and sodium bi-phosphate, Immersion oil and hand wash.

3.3 Methods

3.3.1 Study design

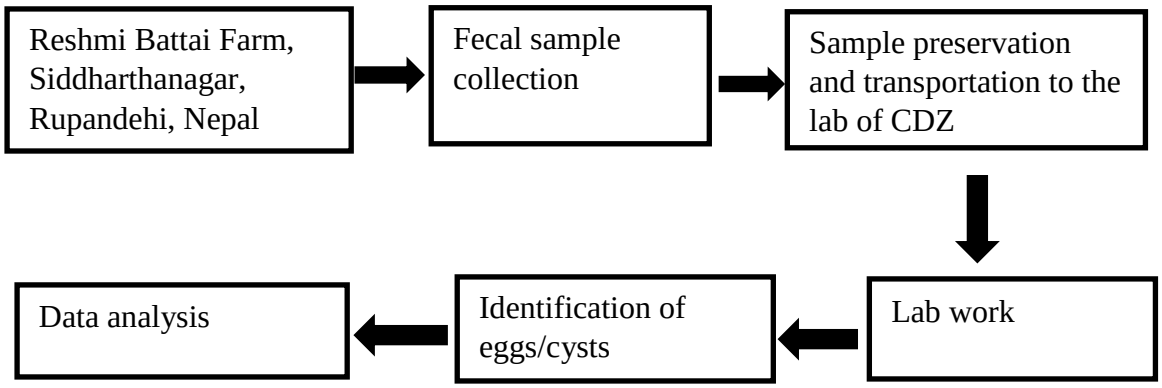

Figure 2: Flow chart showing study design.

3.3.2 Study period

The study was conducted at the Reshmi Battai Farm in Siddharthanagar, Rupandehi, Nepal, where fecal samples were collected from December 2021 to May 2022.

3.3.3 Sample size and collection of fecal samples

A total of 150 fecal samples (75 samples during winter and remaining samples during summer) were collected from the quail's cloaca early in the morning and tagged in their legs to identify that the fecal samples had already been collected from those quails. The vial was labeled with the quail's identification number and the date of collection. All samples collected were correctly labeled.

3.3.4 Preservation of fecal samples

After collecting the sample, it was preserved in a 2.5 percent potassium dichromate ($K_2Cr_2O_7$) solution to preserve the morphology of the eggs and to prevent further development of helminth eggs and larva.

3.4 Sample examination

After collecting samples, it was carried to the laboratory of Central Department of Zoology, kritipur for examination of parasitic eggs and larva. The fecal samples were examined under microscope for trophozoite, cysts, oocysts, eggs, and larvae of gastro-intestinal parasites by using both direct smear and concentration methods (floatation and sedimentation technique). The slides were observed under low power first at 10X and followed to high power at 40X of the microscope.

3.4.1 Direct smear method

3.4.1.1 Saline wet mount examination

A small number of fecal samples were taken with the help of a toothpick and emulsified with normal saline on the clean glass slide. Then a cover slip was placed over it and excess fluid was removed with cotton or filter paper. The smear was observed under the microscope to demonstrate helminthic eggs and larvae (Zajac and Conboy, 2012).

3.4.1.2 Iodine wet mount

A portion of the stool sample was placed on the glass slide and mixed with a drop of Gram's iodine. The mixture was then covered with a cover slip, and excess fluid was removed with cotton filter paper. The smear was examined under a microscope. Iodine wet mount was necessary for the identification and study of the nuclear character of protozoan cysts and trophozoites (Zajac and Conboy, 2012).

3.4.2. Concentration methods

The two concentration methods for routine stool sample evaluation are flotation and sedimentation. Floatation mainly reveals the protozoan and sedimentation reveals helminthic parasites (Arora, 2012).

3.4.2.1 Floatation method

Saturated salt solution was used for this method. The floatation method is used to float the parasite with a density less than that of saturated salt (Arora, 2012).

2gm of sample was filtered and mixed with normal saline in a 15 ml centrifuge tube and then centrifuged at 1200 rpm for 5 minutes. The supernatant was discarded, and a few 4-5 ml of floatation solution was left in the tube. It was mixed well to resuspend the particles. Further concentrated Nacl solution was added and filled the tube up to 14 ml and centrifuged again at 1200 rpm for 5 minutes. The tube was further added with concentrated Nacl drop by drop until a convex surface was formed at the top. A clean cover slip was placed over the top of the tube avoiding any bubbles and was left undisturbed for at least 10 minutes. The cover slip was removed gently avoiding the dropping of the sample from cover slip and then placed over a clean glass slide. The slide was observed under compound microscope both with and without lugol's iodine.

3.4.2.2 Formalin Ethyl Acetate Sedimentation Method

The Sedimentation method reveals the parasites with density higher than the density of the solution. It mainly detects trematode eggs, however some nematode eggs and larva and some cestodes eggs are also detected by this method as they do not float on concentration solution (Arora 2012).

About 2 gm of sample was filtered thoroughly and mixed with normal saline in a 15ml centrifuge tube. The sample was centrifuged at 1200 rpm for five minutes. The supernatant was discarded, and the sediment was mixed well. Then 10ml of 10% buffered formalin and 3ml of ethyl acetate was added in the tube and again centrifuged. Four layers of ethyl acetate, plug of debris, 10% formalin and sediment were formed. The plug of debris was made free with wooden applicator stick then all the supernatant fluid was decanted and discarded. Before bringing the tube to upright position ethyl acetate was made sure to be removed as it forms extensive bubbles under the microscope. In case if sediments were too dry one-two drops of 10% formalin was added and mixed well. A drop of sediment was placed on a clean slide, covered with cover slip and observed under microscope both with and without lugol's iodine.

3.4.3 ZN (Zeihl-Neelsen) acid fast staining

The sediments obtained following the formalin ethyl acetate method were used to prepare thin smear on clear and dry glass slides. The smears were dried at room temperature and fixed with gentle heat. The smear was flooded with carbol Fuchsin stain (S005) and heated to steam for 5 minutes with a low flame making sure the stain was not boiled and dried. Then the slide was allowed to stand for 5 minutes without further heating and washed in running tap water. The stain was decolorized with Acid Fast Decolorizer (S033) for 2 minutes or until no more stain came off in the washing. (If washing was not thorough, there was chance for false positive result). The slide was again washed with water and counterstained for 30 minutes with Methylene blue (S022). Finally, the slide was washed with tap water, dried in air and then examined under oil immersion objective (Henriksen and Pohlenz 1981).

3.5 Eggs and cysts size measurement

The size of the eggs and cysts were measured by using ocular and stage micrometer with calibration factors (C.F).

C.F= (No. of S.D/ No. of O.D) × 10 µm

C.F for 10x= 10 µm

C.F for 40x= 2.6 µm

3.6 Eggs, cysts, and larva size identification:

Cysts, eggs, and larvae of parasites were identified based on morphological characters (shape and size) by using books of (Soulsby 1982, Zajac & Conboy 2012), other published and unpublished article and from internet sources.

3.7 Data analysis

The data was recorded based on a laboratory examination. Microsoft Excel was used to examine recorded data. Additionally, pie charts and bar diagrams were used. The prevalence and concurrency of gastrointestinal parasites were statistically analyzed using the Chi-square test performed by "SPSS software". In each case, 95% C.I and $P< 0.05$ were used to determine whether a difference was statistically insignificant. Prevalence was calculated using a percentage. The Prevalence was computed using the following formula:

Prevalence of parasite= number of stool sample found positive with the parasite /total number of stool samples analyzed

4. RESULTS

4.1 General prevalence of GI parasites in quail

0ut of 150 fecal samples examined, 109 (72.67%) fecal samples were positive with one or more specific GI parasites.

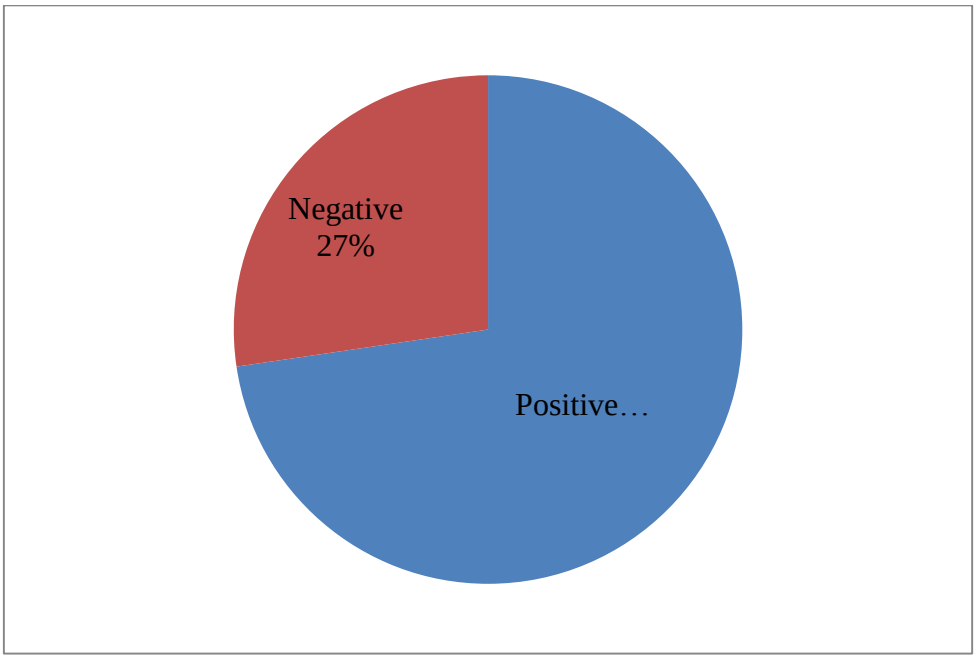

Figure 3: General prevalence of gastrointestinal parasites in quail

4.2 Prevalence of specific GI parasites in quail

Out of 150 fecal samples examined, six different parasites were identified. These parasites included four nematodes, one cestode and one protozoan parasite. *Eimeria* sp. had the highest prevalence (29.33%), followed by *Ascaridia* sp. (21.33%), *Heterakis* sp. (16%), *Capillaria* sp. (11.33%), *Strongyloides* sp. (7.3%), and *Raillietina* sp. (4.6%).

Table 1: Overall prevalence of specific GI parasites in quail

S. N	Class	Parasite name	Positive sample (n=150)	Prevalence (%)
1.	Nematodes	*Ascaridia* sp.	32	21.33%
		Heterakis sp.	24	16%
		Capillaria sp.	17	11.33%
		Strongyloides sp.	11	7.3%
2.	Cestode	*Raillietina* sp.	7	4.6%
3.	Protozoan	*Eimeria* sp.	44	29.33%

4.3 Age-wise prevalence of GI parasites in quail

The age-wise prevalence of GI parasites in quail was categorized into young (Below 1 month) and adult (above 1 month). Out of 150 fecal samples, eighty-five were adults and the rest were young.

Table 2: Age-wise prevalence of gastrointestinal parasites in quail

Determinant	Parameter	Quail examined (n=150)	Quail Infected	Prevalence (%)	$\chi 2$	p-value
Age	Young	65	51	78.46%	1.939	0.164
	Adult	85	58	68.23%		

Table 2 showed age wise (78.46%) young and (68.23%) adults were found to be infected with one or more parasites and was found statistical in significant difference in age wise prevalence of GI parasites in quail ($\chi 2$ =1.939; p>0.05).

4.3.1 Age-wise comparative prevalence of specific GI parasites in quail

Table 3: Age-wise comparative prevalence of specific GI parasites in quail

Class	Parasite name	Age	
		Young (n=65)	**Adult (n=85)**
Nematodes	*Ascaridia* sp.	17 (26.15%)	15 (17.64%)
	Heterakis sp.	13 (20%)	11 (12.94%)
	Strongyloides sp.	4 (6.15%)	7 (8.23 %)
	Capillaria sp.	9 (13.84%)	8 (9.41%)
Cestode	*Raillietina* sp.	2 (3.08%)	5 (5.88%)
Protozoa	*Eimeria* sp.	20 (30.77%)	24 (28.24%)

The study found that *Eimeria* sp., *Ascaridia* sp., *Heterakis* sp. and *Capillaria* sp. were more prevalent in Young quails compared to adults, while *Strongyloides* sp. and *Raillietina* sp. were more prevalent in adults than in young quails. Statistically, the difference in age-wise prevalence of specific GI parasites in quail was found to be insignificant ($\chi 2$= 13.106, p>0.05).

4.4 Season-wise prevalence of GI parasites in quail

The prevalence of GI parasites in quail was categorized by season, specifically winter and summer. Out of 150 fecal samples, 75 were collected during winter season, while the remaining fecal samples were collected during summer.

Table 4: Season-wise prevalence of gastrointestinal parasites in quail

Determinant	Parameter	Quail examined (n=150)	Quail Infected	Prevalence (%)	$\chi 2$	p-value
Season	Summer	75	50	66.67%	2.719	0.99
	Winter	75	59	78.16%		

The study showed season wise (78.16%) winter and (66.67%) summer were found to be infected with one or more parasites and was found statistically insignificant difference in season wise prevalence of GI parasites in quail ($\chi 2 = 2.719$; $p>0.05$).

4.4.1 Season-wise comparative prevalence of specific GI parasites in quail

Table 5: Season-wise comparative prevalence of specific GI parasites

Class	Parasite name	Season	
		Winter (n=75)	Summer (n=75)
Nematodes	*Ascaridia* sp.	14 (18.67%)	18 (24%)
	Heterakis sp.	9 (12%)	15 (20%)
	Strongyloides sp.	7 (9.33%)	4 (5.33%)
	Capillaria sp.	10 (13.33 %)	7 (9.33%)
Cestode	*Raillietina* sp.	5 (6.67%)	2 (2.67%)
Protozoa	*Eimeria* sp.	25 (33.33%)	19 (25.33%)

The study showed that *Eimeria* sp., *Capillaria* sp., *Strongyloides* sp., and *Raillietina* sp. were all found to be more prevalent in winter, while *Ascaridia* sp. and *Heterakis* sp. were more prevalent in summer. Statistically, the difference in season-wise prevalence of specific GI parasites in quail was found to be insignificant ($\chi 2=7.118$, $p>0.05$).

4.5 Types of infection

Out of 150 samples, single infection (52%) was higher than mixed (20.67%). Statistically, the prevalence of single and mixed infection was found to be insignificant difference ($\chi 2=3.458$, $p>0.05$). Since quails are prone to one or more GI parasites at same time, the prevalence was noted for single infection followed by mixed infection.

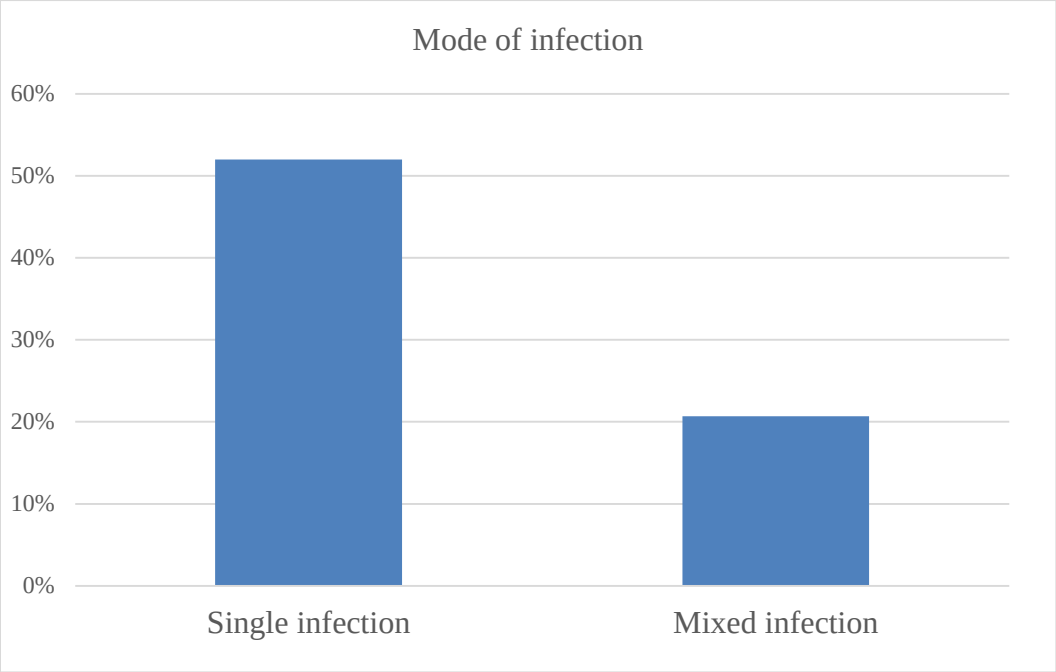

Figure 4: Concurrency of parasitic infection in quails

4.6 Photo plates of Eggs and cysts of GI parasites in quail under microscope at a total magnification (10x*40x)

Photo1: Oocyst of *Eimeria* sp. (20µm/18µm)

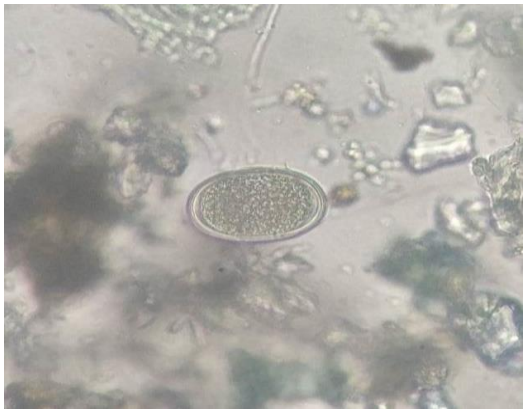

Photo 2: Egg of *Ascaridia* sp. (70µm/54µm)

Photo 3: Egg of *Strongyloides* sp. (55µm/24µm)

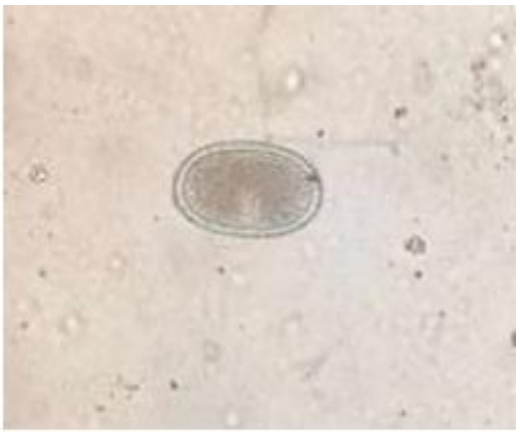

Photo 4: Egg of *Heterakis* sp. (66µm/41µm)

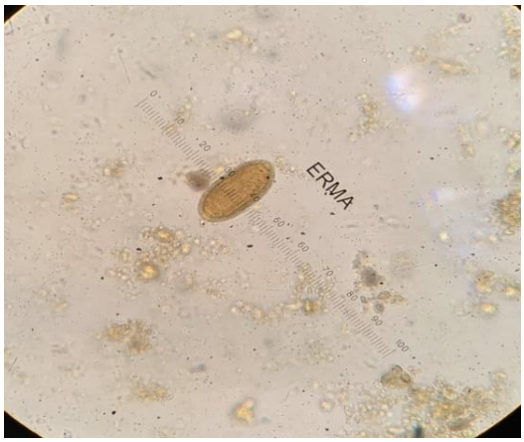

Photo5: Egg of *Capillaria* sp. (59µm/34µm)

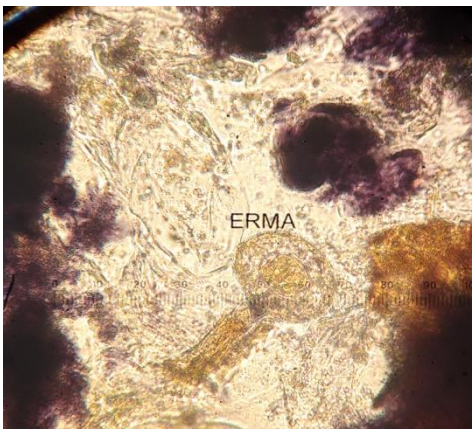

Photo 6: Egg of *Raillietina* sp. (50/25µ)

5. DISCUSSION

Quails were considered more resistant to infections compared to chicken, have become susceptible to a wide range of potentially harmful organisms, including bacteria, viruses, and parasites due to factors like poor management practices, intensive rearing stress, and poor hygiene (Monte et al. 2018). Several studies conducted worldwide concluded that many factors such as bird age, infective dose, environmental conditions, and poor management practices contribute to an increased likelihood of helminthic and protozoan infections in quails (D'souza et al. 2022). In the present study, the prevalence of gastrointestinal parasites was recorded based on age, season, and the occurrence of concurrent mixed infections.

A total of 150 fecal samples have been amassed in this study, of which 72.67%were found to be positive with different groups of gastrointestinal parasites. Compared to previous studies, the prevalence rated in our study was lower than that reported 75.26% in Egypt (El Shabrawy et al. 2016) and 85.2% in Nigeria (Onyeabor & Uwalaka 2021), but higher than rates observed 17.5% in Bangladesh (Islam et al. 2020), 36.66% in southern India (D'souza et al. 2022) and 64.54% in Iraq (AlZarkoushi & AlZubaidi 2021). The difference might be due to a variety of factors such as location, climate, management practices, age, sex, seasons, study methods and sample size.

In this study conducted on quail, six gastrointestinal parasitic infections were observed, including four nematodes, one cestode and one protozoon. Among these parasites, the highest prevalence rate was found in *Eimeria* sp. (29.33%). Similar results were reported in previous studies conducted in Puducherry, India (D'souza et al. 2022) and Egypt (El Shabrawy et al. 2016) where *Eimeria* sp. exhibited high prevalence rates of 23.33% and 42.1% respectively. This indicates that *Eimeria* sp. infection is the most common disease caused by a protozoan that is becoming a problem for poultry globally. This might be related to the ineffective management of litter in quail farms, host immunity status and environmental conditions, which favors the spread of *Eimeria* sp. and quail infection. The remaining parasites, namely *Ascaridia* sp., *Heterakis* sp., *Capillaria* sp., *Strongyloides* sp. and *Raillietina* sp. showed the prevalence of 21.33%, 16%, 11.33%, 7.3% and 4.6% respectively in this study.

In this study, the prevalence of helminth infection (60.56%) was found to be higher than that of protozoan infection (29.33%). This finding is consistent with the results of other studies conducted in Nigeria by (Onyeabor & Uwalaka 2021),Brazil by (Rosa et al. 2017) and Chandragiri Municipality, Kathmandu by (Shrestha et al. 2020), which all demonstrated a higher prevalence of helminthic infections compared to protozoan infections in quails and ducks respectively. Specifically, Onyeabor & Uwalaka (2021) reported a higher prevalence in helminth of 55.6% compared to a protozoan of 29.6% in quails, while Rosa et al. (2017) found higher prevalence in helminths (56%) than protozoa (40%) in quails. Similarly, Shrestha et al. (2020) observed a higher prevalence in helminthic infection (72.84%) than protozoan infection (25%) in ducks. The lower presence of protozoan infection may be attributed to their shorter life cycle when compared to helminths.

Two groups of helminths were identified in quail, including four nematodes and one cestode in the present study. The prevalence of nematodes (55.96%) was found to be higher than that of cestodes (4.6%). This finding agrees with the research conducted by (D'souza et al. 2022) in Puducherry, India, where nematodes (11.67%) were reported to be more prevalent than cestodes (1.6%) in quails. Similar results have been observed in poultry birds in India (Naphade 2013), turkeys in Nigeria (Jegede et al. 2019), wild birds in Nigeria (Assam et al. 2020), ducks in Chandragiri Municipality, Kathmandu (Shrestha et al. 2020) and domestic poultry in Colombia (Montes-Vergara et al. 2021). The difference in prevalence might be attributed to the fact that nematodes do not require intermediate hosts like cestodes do and are primarily transmitted through the soil. Their eggs can remain viable for a long time that consumes from the contaminated environment's bird droppings on a regular basis, increasing parasite burden (Permin et al. 1999). Therefore, factors such as excessive fertility and inadequate sanitation could serve as significant sources of nematode infections.

Among four nematodes, the prevalence rate of infection was found higher in *Ascaridia* sp. (21.33%) followed by *Heterakis* sp. (16%), *Capillaria* sp. (11.33%) and *Strongyloides* sp. (7.3%) in this study. Similar comparatively high prevalence rate of *Ascaridia* sp. over *Heterakis* sp., *Capillaria* sp. and *Strongyloides* sp. has been reported in study of (Onyeabor & Uwalaka 2021) and (D'souza et al. 2022) also showed high prevalence rate of *Ascaridia* sp. over *Capillaria* sp. from quails. This

suggests *Ascaridia* sp. is the most common nematode parasite affecting poultry, possibly due to a host specific relationship between quails and *Ascaridia* sp. Furthermore, Infestation with *Ascaridia* sp. has been known to cause tissue damage in birds by interfering with nutrient absorption, leading to poor growth, reduced production, and in severe cases, intestinal blockage and even death (Das et al. 2015).

The prevalence rate of *Heterakis* sp. is found 16% in this study is consistence with the finding of (Chaudhary 2017) and (Shrestha et al. 2020) who reported rates of 18% and 15% respectively in ducks. However, the observed parasitic positive rate varied significantly across different studies. For instance, high 33.75% observed in study of (Hembram et al. 2015) recorded a considerably higher rate of 33.75% in India, while lower rates of 13.6%, 12% and 7% were observed by (Onyeabor & Uwalaka 2021) in quails, (Khadka 2019) in Kadaknath and (Islam et al. 2020) in quails respectively. These variations in prevalence rate could potentially be attributed to differences in environmental conditions, diet and management practices employed in the respective studies.

In the current investigation, the infection of rate of *Capillaria* sp. was found 11.33% which is consistence with 11% reported by (Onyeabor & Uwalaka 2021) from Nigeria. However, it is higher than 2% reported by (Islam et al. 2020) from Bangladesh and 5% (D'Souza et al. 2020). Different species of *Capillaria* namely *Capillaria anatis* (0.5%), *Capillaria annulate* (3.1%) and *Capillaria contorta* (7.3%) were reported by (Muhairwa et al. 2007) from Tanzania in ducks. Similarly, *Capillaria contorta* (36.83%) and *Capillaria annulata* (21.83%) were observed in previous study conducted by (Paul et al. 2015) in Gombe. The lower prevalence observed in this study could potentially be attributed to variations in rearing practice, environmental conditions, host availability and diet.

Regarding the infection of *Strongyloides* sp., the prevalence rate of 7.3% in the present study was lower than the rates of 15.56% reported by (Paudel 2012) and 12.50% reported by (Shrestha et al. 2020) in ducks. However, it was higher than 3.3% reported by (Onyeabor & Uwalaka 2021) from Nigeria in quails. This difference could be due to ineffective management of litter in quail farms and variations in environmental conditions.

In this study, only one species of cestode i.e., *Raillietina* sp. was recorded with a prevalence of 4.6%. The prevalence was lower than 5% reported by (Shemshadi et al. 2014) and higher than the prevalence rates of 1.6% reported by (D'Souza et al. 2020) from Puducherry, India and 4.18% reported by (Elmorsy et al. 2020). Other species of cestodes namely *Choanotaenia infundibulum and Hymenolepis* sp. were observed in study conducted by (Mohamed et al. 2011). These variations in prevalence rates could be influenced by environmental factors, the availability of intermediate hosts and differences in management practices.

There were no trematodes reported in this study. This finding is supported with the previous studies conducted by (Kumar et al. 2003) in India, (Onyeabor & Uwalaka 2021) in Nigeria, (D'souza et al. 2022) in Puducherry, India (El Shabrawy et al. 2016) in Egypt and (Shemshadi et al. 2014) in Iran. The reasons for absence of these parasites in the quails might be related to the complex life cycle that requires at least one intermediate host that is aquatic. Since, quails were reared inside a coop where they were totally out of contact with such hosts.

In the present study, single parasitic infections were found to be more common in quails (52%) compared to mixed infections (20.67%). This finding is consistent with a study by (Shrestha et al. 2020) conducted in ducks from Chandragiri municipality, Kathmandu, Nepal, where 65% of ducks had single infection followed by 16.67% with mixed infection. Similar studies were observed by (Dauda et al. 2016) in domestic turkey, where 47.50% had single infection followed by 26% with mixed infection, (Ogbaje et al. 2012) where 37.5% had single infection followed by double infection (23.9%) and 13.6% had the triple infection and (Mohamed et al. 2011) in quails. This could be due to various factors such as farm management practices or food choices at a specific time, which influenced the development of a mixed or single infection in the host. The coexistence of two or more parasites in the same host can increase the prevalence of mixed infections. However, as the number of parasites per host increases, the overall prevalence tends to decreases because parasites may not able to tolerate each other (Smyth 1976).

The prevalence of gastrointestinal parasites was observed to be higher in young quails (78.46%) compared to adults (68.23%) in this study. This finding is consistent with studies conducted by (D'Souza et al. 2022) in Puducherry, India, (Arafat & Abbas

2018) in Egypt, (Mohammad 2012) in Iraq and (Bashtar et al. 2010) in Saudi Arabia. Furthermore, the prevalence of helminths was higher in young quails (69.22%) than in adults (54.1%) and the prevalence of protozoa was also higher in young quails (30.77%) compared to adults (28.24%) in this study. Similar result were reported by (Onyeabor & Uwalaka 2021) in Nigeria. This could be because young quails are more vulnerable, or it could be due to the development of immunity in adult quails.

In this study, the prevalence of gastrointestinal parasites were found to be higher in the winter season (78.67%) compared to the summer season (66.67%). Similar finding was reported by (AlZarkoushi & AlZubaidi 2021), who found a higher prevalence in winter (70.70%) than in summer (51.51%), (Hassan et al. 2020) also reported a higher prevalence rate in the cold season (57%) compared to the warm season (53%), while (Islam et al. 2016) revealed a higher prevalence rate in winter (2.10%) compared to summer (1.26%). This could be due to various factors such as poor management practices during the cold season, high atmospheric humidity, and litter moisture which creates favorable conditions for the survival and transmission of gastrointestinal parasites.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

From this study, it is concluded that quails of Reshmi Battai Farm from Siddharthanagar, Rupandehi, Nepal were infected with different GI helminths and protozoan parasites. The common GI parasites were *Eimeria* sp., *Ascaridia* sp. *Heterakis* sp., *Capillaria* sp., *Strongyloides* sp. and *Raillietina* sp. Among identified GI parasites, *Eimeria* sp. was found to be the most prevalent. There were no trematodes found in this study. The prevalence of GI parasites in quails is high and does not differ significantly by age or season, with most quails having single parasite infections. The results indicate that quails are highly susceptible to gastrointestinal parasites and need to undertake preventive measures for controlling the risk of parasitosis in quail.

6.2 Recommendations

Based on outcome of the present study, following measures are recommended to reduce the risk of gastro-intestinal parasitic infection of quails are:

- As quails are infected with both helminth and protozoan parasites, good and hygienic management practices as well as appropriate anthelminthic and antiprotozoal treatments should be done.
- A sex wise study can be studied.
- Further study based on molecular techniques should be done for the identification of parasitic species.

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ANNEX

Photo 7: Quails

Photo 8: Sample collection

Photo 9: Samples

Photo 10: Sample Processing

Photo 11: Slide for observation

Photo 12: Sample Observation