

**PREVALENCE OF GASTROINTESTINAL PARASITES OF PIGEON
(*Columba* sp. Linnaeus, 1758) IN THREE TEMPLES OF POKHARA
VALLEY**



Amrit Gurung

T.U. Registration No. : 5-2-48-2546-2007

T.U. Examination Roll. No. : 4

Batch : 2070/071

A thesis submitted in partial fulfillment of the requirements for the award of the degree of
Master of Science in Zoology with special paper Parasitology

Submitted to

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

December, 2016

DECLARATION

I hereby declare that the work presented in this thesis 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).

Date:

.....

Amrit Gurung



TRIBHUVAN UNIVERSITY

01-4331896

CENTRAL DEPARTMENT OF ZOOLOGY

Kirtipur, Kathmandu, Nepal.

Ref.No.:

RECOMMENDATION

This is to recommend that the thesis entitled "**Prevalence of Gastrointestinal Parasites of Pigeon (*Columba sp. Linnaeus, 1758*) in Three Temples of Pokhara Valley**" has been carried out by Amrit Gurung for the partial fulfillment of Master's Degree of Science in Zoology with special paper Parasitology. This is his 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.

Date:

.....

Supervisor

Mr. Janak Raj Subedi

Lecturer

Central Department of Zoology

Tribhuvan University

Kirtipur, Kathmandu, Nepal



TRIBHUVAN UNIVERSITY

☎ 01-4331896

CENTRAL DEPARTMENT OF ZOOLOGY

Kirtipur, Kathmandu, Nepal.

Ref.No.:

LETTER OF APPROVAL

On the recommendation of supervisor "**Mr. Janak Raj Subedi**" this thesis submitted by Amrit Gurung entitled "**Prevalence of Gastrointestinal Parasites of Pigeon (*Columba* sp. Linnaeus, 1758) in Three Temples of Pokhara Valley**" is approved for the examination and submitted to the Tribhuvan University in partial fulfillment of the requirements for Master's Degree of Science in Zoology with special paper Parasitology.

Date:

.....

Prof. Dr. Ranjana Gupta
Head of Department
Central Department of Zoology
Tribhuvan University
Kirtipur, Kathmandu, Nepal



TRIBHUVAN UNIVERSITY

01-4331896

CENTRAL DEPARTMENT OF ZOOLOGY

Kirtipur, Kathmandu, Nepal.

Ref.No.:

CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Amrit Gurung entitled "**Prevalence of Gastrointestinal Parasites of Pigeon (*Columba* sp. Linnaeus, 1758) in Three Temples of Pokhara Valley**" has been approved as a partial fulfillment for the requirements of Master's Degree of Science in Zoology with special paper Parasitology.

EVALUATION COMMITTEE

.....

(Supervisor)

Mr. Janak Raj Subedi

Central Department of Zoology

Tribhuvan University

Kirtipur, Kathmandu, Nepal

.....

(Head of Department)

Prof. Dr. Ranjana Gupta

Central Department of Zoology

Tribhuvan University

Kirtipur, Kathmandu, Nepal

.....

External Examiner

.....

Internal Examiner

Date:

ACKNOWLEDGEMENTS

I would like to express my heartfelt gratitude to my supervisor Mr. Janak Raj Subedi, Lecturer, Central Department of Zoology, T.U. Kirtipur for his supervision, guidance and invaluable suggestion throughout my study.

I am thankful to our honorable Head of Department Prof. Dr. Ranjana Gupta, Central Department of Zoology, T.U. Kirtipur for providing me such an opportunity to carry out this dissertation work.

I am indebted to my friends: Bidur Gurung, Ganesh Ghimire, Sandesh Gurung, Naresh Oli, Pujan Adhikari, Rishi Baral, Bishnu Achhami, Prabin Baral and Nirmala G.C. for their constant help during study period.

Heartly thanks to my family without which this work is impossible to complete.

I also acknowledge to all the teachers, friends and staffs of Central Department of Zoology for their continuous aspiration and motivation.

Amrit Gurung
Symbol No: 4
Batch: 2070/071
aaritha.grg@gmail.com

CONTENTS

	Pages
Declaration	i
Recommendation	ii
Letter of approval	iii
Certificate of acceptance	iv
Acknowledgements	v
Contents	vi-vii
List of table	viii
List of figures	viii
List of photographs	viii
List of abbreviations	ix
Abstract	x
1. INTRODUCTION	1-4
1.1 Background	1-2
1.2 Pigeon, human and parasites in nature	2
1.3 Parasitic infections	2-3
1.4 Objectives	3
1.4.1 General objective	3
1.4.2 Specific objectives	3
1.5 Justification of the study	3-4
1.6 Limitation of the study	4
1.7 Hypothesis	4
2. LITERATURE REVIEW	5-8
2.1 In global context	5-8
2.2 In context of Nepal	8
3. MATERIALS AND METHODS	9-13
3.1. Study area	11
3.2 Materials used	11
3.2.1 Equipments	11
3.2.2 Chemicals	11
3.3 Study design	11-12
3.3.1 Sample collection method	11
3.3.2 Preservation of faecal samples	11
3.3.3 Sample size	12
3.4 Interview	12
3.5 Laboratory examination	12-13
3.5.1 Iodine wet mount	12

3.5.2 Concentration techniques	12
3.5.2.1 Floatation technique	12
3.5.2.2 Sedimentation technique	12-13
3.5.3 Eggs and cysts size measurement	13
3.5.4 Eggs and cysts identification	13
3.6 Data analysis	14
4. RESULTS	15-17
4.1 General prevalence of GI parasites	15
4.2 Prevalence of specific GI parasites	15
4.3 Prevalence of protozoan and helminth parasites	16
4.4 Area wise prevalence	16
4.5. Infection-wise prevalence	17
4.6 Health care	17
5. DISCUSSION	20-23
6. CONCLUSION AND RECOMMENDATIONS	24
6.1 Conclusion	24
6.2 Recommendations	24
7. REFERENCES	25-28

LIST OF TABLE

Table	Title of Table	Page
1.	Identification characters of egg and cyst of parasites.	13

LIST OF FIGURES

Figure	Title of figures	Pages
1.	General prevalence of GI parasites.	15
2.	Prevalence of specific GI parasites	15
3	Prevalence of protozoan and helminth parasites.	16
4.	Area-wise prevalence	16
5.	Infection-wise prevalence	17

LIST OF PHOTOGRAPHS

Photograph	Title of photograph	Pages
1.	Map of Pokhara valley showing study area.	10
2.	Sample collection at Bindhyabasini temple	18
3.	Sample collection at Bhadrakali temple.	18
4.	Sample collection at Tal Barahi temple.	18
5.	Sample preservation.	18
6.	Sample examination (centrifugation).	18
7.	Sample examination (concentration technique).	18
8.	Sample examination.	18
9.	<i>Ascaridia</i> sp.	19
10.	<i>Capillaria</i> sp.	19
11.	Coccidia	19
12.	<i>Echinostoma</i> sp.	19
13.	<i>Heterakis</i> sp.	19
14.	<i>Hymenolepis</i> sp.	19
15.	<i>Syngamus</i> sp.	19

LIST OF ABBREVIATIONS

µm	- Micrometre
BCN	- Bird Conservation Nepal
CI	- confidence interval
DNPWC	- Department of National Parks and Wildlife Conservation
EPG	- Egg per gram
GI	- Gastro Intestine
NaCl	- Sodium Chloride
RH	- Relative Humidity
viz.	- namely

ABSTRACT

The present study was conducted to determine the general prevalence, identification, compare area-wise as well as infection-wise prevalence and find out activities on health care of pigeon (*Columba* sp.) in three temples viz. Bhadrakali temple, Bindhyabasini temple and Tal Barahi temple of Pokhara valley. A total of 120 faecal samples were collected by opportunistic random faecal sampling method on 16, 17 and 19 March, 2016 A.D. Iodine wet mount and different concentration technique (floatation and sedimentation) were used for faecal qualitative tests and verbally administered questionnaires for interview whereas Microsoft Excel 2007 and “R”, version 3.3.1 software packages were used in analyzing data. Out of 120 faecal samples examined, 83 faecal samples were positive with 69.16% prevalence of parasitic infection. Total of seven GI parasites that includes one subclass of protozoan: *Coccidia* 23 (19.16%) and six genera of helminths: *Capillaria* sp. 38 (31.67%), *Ascaridia* sp. 26 (21.66%), *Echinostoma* sp. 9 (7.50%), *Syangamus* sp. 7 (5.83%), *Hymenolepis* sp. 4 (3.33%) and *Heterakis* sp. 3 (2.50%) were identified and reported first time in Nepal. The prevalence rate of helminths 66 (55%) were higher than protozoan parasites 23 (19.16%). The higher prevalence of GI parasites was in Bhadrakali temple 31 (77.50%) followed by Tal Barahi temple 29 (72.50%) and the lowest was in Bindhyabasini temple 23 (57.50). Statistically, the difference in prevalence of GI parasitic infection among study area was found to be insignificant ($\chi^2=0.76328$, $P>0.05$) whereas the difference in prevalence of single infection 52 (43.83%) and mixed infections 31 (25.83%) were insignificant ($\chi^2=3.4728$, $P>0.05$). No any activities on health care of pigeon regarding the GI parasites were found. The study indicated that pigeons in three temples of Pokhara valley were highly susceptible to GI parasites. Therefore sustainable ways for controlling the parasitic infection and further studies need to be designed for the health and conservation of pigeons.

1. INTRODUCTION

1.1 Background

Pigeons (Order Columbiformes) are ubiquitous birds and can be found virtually in every town and city around the globe (Marques *et al.*, 2007). Pigeons are related to human since ancient time (BC. 3000-5000). They live side by side with human as a source of food, hobby, experimental purpose, cultural and religious symbols (Sari *et al.*, 2008). *Columba livia* Gmelin, 1789 are descended from wild rock pigeons that live in Mediterranean Europe (Adang, 1999). The intensive and divergent selection that has gone into the establishment of these innumerable breeds and varieties has resulted in a great variance in conformation, feathering, colour and behavior, since mid-century (Burger, 1974). About 100 different breeds and varieties are described in more or less detail (Levi, 1969). In Nepal, six species of *Columba* are recorded: *Columba livia*, (*Columba rupestris* Pallas, 1811), (*Columba leuconata* Vigors, 1831), (*Columba palumbus* Linnaeus, 1758), (*Columba hodgsonii* Vigors, 1832) and (*Columba pulchricollis* Blyth, 1846) (BCN and DNPWC, 2016).

Pigeons are primarily grain and seed eaters and will subsist on spilled or improperly stored grain. They will also feed on garbage, livestock manure, insects or other food materials provided for them intentionally or unintentionally by people (Williams and Corrigan, 1994). Food consumption is about one-tenth of the pigeon's body weight and will range from 20-100 gm daily, depending on the strain (Sturtevant and Hollander, 1978). Pigeon consumes 36-60 ml of water daily (Clarkson *et al.*, 1963). They rely mostly on free-standing water but they can also use snow to obtain water. In fact, in some urban areas the feeding of pigeons is considered a form of recreation (Williams and Corrigan, 1994).

Pigeons are monogamous. Eight to 12 days after mating, the females lay one or two eggs which hatch after 18 days. The male provides nesting material and guards the female and the nest. The young are fed pigeon milk, a liquid solid substance secreted in the crop of the adult (both male and female) that is regurgitated. The young leave the nest at four to six weeks of age. More eggs are laid before the first clutch leaves the nest. Average weight of pigeon is 369 gm and the average length is 11 inches. Breeding may occur at all seasons, but peak reproduction occurs in the spring and fall (Williams and Corrigan, 1994). They breed well for at least five to six years and will continue to reproduce, but less regularly into an old age of 10 or more years. Young pigeons reach sexual maturity by six to seven months (Hollander, 1954; Levi, 1969; Sturtevant and Hollander, 1978; Kendall and Scanlon, 1981). A population of pigeons usually consists of equal numbers of males and females. In captivity, pigeons commonly live up to 15 years and sometimes longer (Williams and Corrigan, 1994).

Pigeons may be raised under wide seasonal ranges of temperature, humidity, light, and barometric pressure, if kept in flypens. For cage housed birds, temperature should be held between 10°-24°C (50°-57°F) with relative humidity (RH) of 30%. A 12-hour light, 12 hour dark diurnal cycle is commonly provided, although 14 hours light will enhance breeding activity (Sturtevant and Hollander, 1978). Pigeons lay successive clutches of

two eggs at five week intervals and raise 10-22 young per annum where 15 to 16 is considered a good commercial production (Levi, 1969; Kendall and Scanlon, 1981; Hollander, 1954; Sturtevant and Hollander, 1978).

1.2 Pigeon, human and parasites in nature

Pigeons breeding and roosting sites host an endless number of arthropods (bugs, fleas, mites and ticks) that may infest humans (Haag-Wackernagel and Bircher, 2009; Mumcuoglu *et al.*, 2005). They have the potential for transmission over 30 diseases to humans plus another ten to domestic animals (Weber, 1979). Several health problems can affect pigeons but parasite infections play a major role. They constitute a major source of infection and transmission of diseases (Marques *et al.*, 2007). The effects of parasitism on birds are often severe, including retarded growth, low egg production and susceptibility to other infections (Dranzoa, 1999). Parasites often have short life-cycles. This gives them a capacity to change in genetic composition between generations and hosts need ability to respond these changes (Lindström, 2000). Birds can be parasitized by a wide variety of ecto and endoparasites that is nematodes, trematodes, cestodes, acanthocephalans (Marques *et al.*, 2007). Pigeons can carry many parasites and pathogens to different flocks. Domestic pigeons don't go for migration, but if allowed they return to their nests from long distance due to their good homing ability (Opara *et al.*, 2012).

Pigeons are often found along with human habitation, occupying and soiling places where people work and stay (Adang, 1999). Pigeons may be infected with many organisms, some of which are pathogenic to humans (Zwart, 1986). Feral pigeons have been identified with mycotic, bacterial, protozoal, chlamydial, rickettsial and parasitic diseases as well as dermatosis have been identified from the transmission of pigeons to humans (Weber, 1979). Those who watch these birds can barely imagine how detrimental their disorderly reproduction may be and how many risks they pose to human health. They constitute a major source of infection and transmission of diseases. They are often a cause for repulsion and nuisance due to the accumulation of faecal droppings and to the disruptive noise associated with their presence. Humans are infected by inhaling faecal dust from cages or from sites that have been contaminated with dry faeces, urine and other droppings. This usually occurs among breeders, veterinary doctors, industrial workers and cleaning workers (Marques *et al.*, 2007).

1.3 Parasitic Infections

Parasites may be responsible for a number of serious health problems among pigeons either directly or indirectly. Avian parasites commonly seen include protozoa (one-celled animals), helminths (worms) and arthropods (insects and mites). The effects vary from benign to acute death (Ritchie *et al.* 1997).

There are more than 2,00,000 named species of protozoa of which nearly 10,000 are parasitic in invertebrates and in almost every species of vertebrate (Collier *et al.*, 1998). As a general rule, protozoa multiply by asexual reproduction. This is not to say that sexual processes are absent in the protozoa. Some parasitic forms may have an asexual phase in one host and a sexual phase in another host. In most parasitic protozoa, the developmental stages are often transmitted from one host to another within a cyst. The

reproduction process is also related to the formation of the cyst. Asexual reproduction of some ciliates and flagellates is associated with cyst formation, and sexual reproduction of sporozoa invariably results in a cyst. Protozoa of medical importance are classified on the base of their morphology and locomotive system (Assafa *et al.*, 2006).

a. Amoeba b. Flagellates c. Ciliophora d. Coccidian

Several helminth have been implicated in causing morbidity and mortality in pigeons (Soulsby, 1982) as well as considered as the greatest impediments to profitable pigeon production (Galloway, 1972). Helminth infections may have particularly deleterious or debilitating effects on infected birds, specially the young birds (squabs) causing retarding growth and interfering healthy development as well as making older birds prone to secondary infections (Cheng, 1973; Adang, 2008).

The sources of the parasites are different. Exposure of parasites may occur in one of the following ways:

- a. Contaminated soil (Geo-helminths), water and food
- b. Blood sucking insects or arthropods
- c. Domestic or wild animals harboring the parasite
- d. Oneself (auto-infection)
- e. Sexual intercourse.

They enter the body through different routes including mouth, skin and the respiratory tract (Assafa *et al.*, 2006). The helminths are classified into three major groups. They are:

a. Cestodes (Tape worms) b. Nematodes (Round worms) c. Trematodes (Flukes)

There is also the possibility that domestic pigeons may serve as alternative hosts for some helminths of poultry with which they interact and closely related phylogenetically (Matur, 2010). The prevalence and intensity of parasite may be influenced by several factors, such as climatic conditions (temperature and humidity) that alter the population dynamics of the parasites, resulting in dramatic changes in the prevalence and intensity of helminths infections (Magwisha., 2002). The life cycle may be direct or indirect including an intermediate host. Some species require a second intermediate host or even a third (Permin and Jorgen, 1998).

1.4 Objectives

1.4.1 General objective

- ❖ To determine the general prevalence of gastrointestinal parasites of pigeon (*Columba* sp. Linnaeus, 1758) in three different temples viz. Bhadrakali temple, Bindhyabasini temple and Tal Barahi temple of Pokhara valley.

1.4.2 Specific objectives

- ❖ To identify the gastrointestinal parasites of pigeon.
- ❖ To compare area-wise and infection-wise prevalence of gastrointestinal parasites of pigeon.
- ❖ To find out activities on health care of pigeon regarding the GI parasites.

1.5 Justification of the study

Limited studies have been carried regarding the parasitic infections of pigeon in Nepal. Almost no work has been done in our country if we compare to other nations. Study was conducted to determine the general prevalence, identification, compare area-wise as well as infection-wise prevalence and find out activities on health care regarding the GI parasites of pigeon in three temples of Pokhara valley. This area is still untouched. This study will reveal different GI parasites, health care activities, health hazard on pigeons specially by GI parasites in three temples viz. Bhadrakali temple, Bindhyabasini temple and Tal Barahi temple of Pokhara valley and helps for the further study.

1.6 Limitation of the study

This research has been carried out for the partial fulfillment of the requirements for the master's degree in Zoology at Tribhuvan University, Kathmandu, Nepal. The study was only limited to certain parameters related to the topic due to resources, cost and time constraints. The identification of parasite's eggs and cyst was limited to morphological basis with light microscopy so identification was not possible up to species level.

1.7 Hypothesis

The null and alternative hypothesis of this research work is:

H_0 =There were no significant differences of gastrointestinal parasites and risk factors.

H_1 =There were significant differences of gastrointestinal parasites and risk factors.

2. LITERATURE REVIEW

Columba sp. are associated with human habitation, often occupying and soiling premises where people work and live (Adang, 1999). Pigeons may be infected with many parasites, some of which are pathogenic to humans (Zwart, 1986).

The common internal parasitic infections occur in birds include cestodes, nematodes and coccidians. Free-range scavenging birds are in direct contact with parasite vectors, soil and faeces. On the other hand, lack of hygiene, direct contact with humans, captivity conditions and the physical environment (rainfall, humidity and ambient temperature) provides optimum conditions to maintain parasites populations. The probability of disease transmission is influenced by many factors, such as time of infection, latent period, stability of the agent when exposed to the environment, population density, animal handling, virulence and route of infection (Alves *et al.*, 2008).

Intermediate hosts of parasites easily infect the *Columba* sp. via their diet. Parasites are the aetiological agents of most diseases of cage and aviary birds. The high parasitic infection means an indication of poor management and control efforts in either the animal or in the immediate environment where infection or re-infection (directly or indirectly) may emanate. Both sexes of pigeons are equally at risk of being infected by the parasites and as carriers of pathogenic organisms, some of which might be zoonotic (Opara *et al.*, 2012).

The pigeons have high antibody titres to the protozoan parasite. The latter infection in domestic pigeons has public health implications. Domestic pigeons that carry the parasites may be considered to be a potential source of infection to pigeon keepers, particularly those who are immune suppressed by HIV/AIDS (Mushi *et al.*, 2000).

2.1 In global context

Routine examination within three to four months after death of 609 Band-tailed Pigeons (*Columba fasciata fasciata* Say, 1822) in Colorado was performed. They revealed that 12.50% harboured helminths that includes two species of cestodes and four species of nematodes. *Hymenolepis armata*, *Raillietina* sp., *Ascaridia columbae*, *Splendido filaria colubensis*, *S.hibleri* and *Chandlerella robinsoni* were helminths found above nine months of age whereas absent in younger than nine months of age (Olsen and Braun, 1980). Similar prevalence rate (23.50%) of helminth that includes cestodes only were found while studying the ecto-, gastro-intestinal and haemo-parasites of live pigeons (*Columba livia*) in Kampala, Uganda by using direct smear, floatation and sedimentation tests. The identification of cestodes was not possible (Dranzoa *et al.*, 1999).

Adang *et al.* (2008) humanely killed, dissected and necropsied 240 *Columba livia domestica* in Zaria area, Nigeria. Among which 48.30% were infected by nine species of helminths, comprising six species of cestodes and three species of nematodes. The higher prevalence of cestodes (*Raillietina tetragona*) was recorded whereas single infection (37.50%) was more common than double (10%) and triple infections (0.83%).

Adang *et al.* (2009) conveyed Speckled Pigeons as a probable definitive host of some ectoparasites and helminths. A total of 30 that comprises 20 males and 10 females

Speckled Pigeons were trapped from the wild in Zaria, Nigeria and cut the GI tract of each bird for test. The prevalence rate of helminths infections was found to be 56.70% represented by four species of cestodes comprising *Raillietina tetragona*, (3.30%), *Raillietina cesticillus* (26.70%), *Amoebotaenia cuneata* (13.30%) and *Hymenolepis carioca* (13.3%). Single infection was the only infection type observed.

Msoffe *et al.* (2010) found prevalence rate (79.50%) of GI helminths in domestic pigeons of Morogoro Municipality, Tanzania by the postmortem examination. The three subfamilies represented two cestodes and one nematode, whereas no trematodes were found. Three species of helminths: *Raillietina tetragona* (6%), *Raillietina echinobothrida* (63%) and *Ascaridia galli* (15.50%) were identified. Nestlings appeared to be less susceptible to GI cestodes but more susceptible to nematodes compared with adults. Radfar *et al.* (2012) performed same examination method for the first survey to determine the prevalence and intensity of parasites among free-range backyard chicken and domestic pigeon (*Columba livia domestica*) in Sistan region. Out of 46 domestic pigeon, 39 (84.78%) were infected with parasites. They found seven species of parasites including two species of nematodes, two species of cestodes and three species of ectoparasites. The parasites were *Ascaridia colombae* (15.21 %), *Hadjelia truncata* (17.39 %), *Raillietina tetragona* (26.08%), *Raillietina echinobothrida* (28.26%), *Argas reflexus* (13.04%), *Menopen gallinae* (32.60%) and *Columbicola Columba* (41.30%).

Bahrami *et al.* (2013) indicated that young pigeons could be more susceptible to parasitic infection as compared to above two years old birds but these parasites did not cause any visible deleterious effects in the blood parameters. This could be due to immune response of the pigeons to parasitic infections. Out of 250 samples, 79.20% were positive and 19.19% were carrying multiple infections when examined through direct smear method. The parasites that have been identified in this study consist *Raillietina* sp. (24.24%), *Capillaria* Zeder, 1800 (14.14%), *Tetramers* (8.08%), *Ascaridia* Dujardin, 1845 (4.04%), *Syangamus* (9.09%), Oocyst protozoa (7.07%), *Phthiraptera* (8.08%), *Ceratophyllus columbae* (6.06%).

Radfar *et al.* (2011) surveyed parasites by postmortem examination of 102 domestic pigeons (*Columba livia domestica*) in a selected semiarid zone of South Khorasan, Iran. They found 42.15% prevalence of helminths including two species of nematodes, *Ascaridia colombae* (16.66%) and *Hadjelia truncate* (1.96%), while 3 species of cestodes, *Cotugnia digonopora* (13.79%), *Raillietina magninumida* (18.62%) and *Raillietina achinobothrida* (32.35%). Borji *et al.* (2012) examined 300 domestic pigeons (*Columba livia*) by same method in Mashhad, Iran region. Among which 21.60% and 15.30% were infected with nematodes and cestodes respectively whereas no trematodes were found. The overall prevalence of helminths recorded was 36.90%. Nematodes found were *Ascaridia columbae* (20.37%), *Capillaria bursata* (3.70%), *Capillaria caudinfillata* (1.85%), *Eulimdana clava* (2.70%), *Gongylostrongylus* spp. (0.90) and cestodes were *Choanotaenia infundibulum* (11.11%) and *Raillietina echinobothrida* (6.48%).

Begum and Sehrin (2012) examined the separated parts of the alimentary canal of the Pigeons taken in 0.85% normal saline solution to collect helminth parasites in pigeon (*Columba livia*). They found all the birds infected by eleven species of helminth parasites. Among which, four species of trematoda found were *Echinostoma revolutum* Froelich,

1802 (15%), *E. trivolvyus* (5%), *Patagifer bilobus* (5%), *Ehinoparyphium recurvatum* (8.33%) whereas six species of cestoda found were *Hymenolepis columbae* (63.33%), *Raillietina echinobothrida* (100%), *R. bonini* (43.33%), *R. cesticillus* (100%), *Cotugnia celebesensis* (68.33%), *C. cuneata* (100%) and one species of nematode found was *Ascaridia columbae* (28.33%). In autumn season highest intensity of infection was found. Nagwa *et al.* (2013) used direct microscopic examination and concentration floatation technique. The seasonal dynamic revealed that the highest incidence of *Eimeria* sp. and *Cryptosporidium* in pigeon was in winter (42% and 3.6%) respectively whereas the lowest rate of protozoa in pigeons was in spring (20.4 and 2.6%) respectively.

Patel *et al.* (2000) found 53.57% positive for parasitic infection, out of 106 faecal samples of pigeon by using sedimentation technique in the laboratory. *Ascaridia* sp. and *Capillaria* sp. were helminth found with 20.75% and 13.20% respectively whereas 17.92% of *Eimeria* sp. was only protozoan found. Sari *et al.* (2008) found similar prevalence rate of parasites with 46.12% when examined 251 faecal sample of pigeons (136 domestic pigeons and 115 wild ones) through the centrifugal floatation and modified acid-fast staining methods. Coccidia Leuckart, 1879 oocysts were detected 59.60% in domestic pigeons and 30.40% in wild pigeons. Helminth eggs detected were 23.50% in domestic pigeons and 4.30% in wild pigeons. The helminth species identified were *Capillaria* sp. (19.90%), *Ascaridia columbae* (5.10%) and *Heterakis* sp. Schrank, 1790 (3.70%) in domestic pigeons whereas *Capillaria* sp. (4.30%) and *Syngamus* sp. Montagu, 1811 (1.70%) in wild pigeons.

Opara *et al.* (2012) examined faecal samples of 150 street pigeons and also found similar 70 (46.70%) prevalence rate of GI parasites. Four GI parasites were identified with *Trichomonas* sp. giving the highest prevalence rate (42%), followed by *Eimeria* sp. (28%) whereas Coccidians and *Ascaridia* sp. returned the least with each having the prevalence rate of 14%.

Marques *et al.* (2007) found 74.14% prevalence rate of GI parasites in urban areas of Lages, Southern Brazil. Protozoa (100% for *Eimeria* sp.) were detected and nematodes (*Ascaridia* sp. and *Capillaria* sp.) 32.56% were detected among positive case. The multiple parasite infections were 20.93%. Gosh *et al.* (2014) also revealed similar prevalence rate (72%) of GI parasitic infection using direct smear, floatation and sedimentation techniques in Chittagong metropolitan area, Bangladesh. They found six different species of parasites, among which highest prevalence was recorded for *Ascaridia galli* (35%) followed by *Capillaria* sp. (22%), *Heterakis gallinarum* (13%), *Eimeria* sp. (11%), *Raillietina* sp. (6%) and *Syngamus trachea* (3%). Similarly same methods were used in YSR Kadapa district of Andhra Pradesh in India (Sivajothi and Sudhakara, 2015). They found 72.70% of the birds harbored parasites including *Ascaridia colombae* (33.30%), *Eimeria* sp. (31%), *Capillaria colombae* (17.40%) and *Raillietina* (9%). *Eimeria* sp. was found to be higher in squabs than compare with adults.

Al-Barwari and Saeed (2012) found 100% parasitic infection when examined blood samples, ectoparasites and alimentary canal. Examination of the alimentary canal of *Columba livia* was done for protozoans and helminths parasite. They found four protozoa species, *Eimeria labbeana*, *Trichomonas gallinae*, *Haemoproteus columbae* and *Plasmodium* sp. whereas eight cestoda species, four of each of the genera *Cotugnia* and

Raillietina and four nematoda species, *Ascaridia columbae*, *A. galli*, *Capillaria obsignata* and *Synhimantus spiralis*.

Mushi *et al.* (2000) observed *Ascaridia columbae* (30%) and *Dispharynx spiralis* (10%), *Raillietina* sp. (80%) and coccidian oocysts (40%) by isolating digestive tract of pigeon. Natala *et al.* (2009) also used similar method for examination of samples and observed *Eimeria* sp. (49.20%), *Haemoproteus columbae* (15.60%), *Leucocytooson* sp. (6.40%) and *Plasmodium relicyum* (0.80%) protozoan parasites whereas *Raillietina terragona* (4.90%), *Raillietina cesticillus* (3%), *Raillietina echinobothrida* (7.60%), *Ascaridia columbae* (1.20%), *Ascaridia galli* (1.20%) and *Capillaria anatis* (0.80%) were the helminths seen. The presence of these parasites was considered to pose a danger to achieving the full potentials of the up-coming pigeon barbecue business in Zaria. Similarly *Echinostoma revolutum* (25%), *Raillietina echinobothridia* (50%) and *Cotugnia cuneata* (25%) were observed by same method (Musa *et al.*, 2011).

Ledwoń *et al.* (2016) diagnosed only two known cases of fluke invasions in racing pigeons (*Columba livia*) originating from different regions of Poland over 4 years. In both cases, the invasion was characterized by a very high mortality (approximately 70%), and the source of the infestation was snails of the Lymnaeidae family eaten by pigeons. Fluke invasions in pigeons are extremely rare. Using molecular biology techniques, infestation with the fluke *Echinostoma revolutum* was determined in the second case.

2.2. In context of Nepal

Limited studies have been carried out in context of Nepal. Using dissection method (Meggitt, 1924) recorded *Raillietina torquata* whereas (Sharma, 1943) recorded *Raillietina kantipura*, *Raillietina nagpurensis* and *Raillietina nripendra* from Kathmandu.

3. MATERIALS AND METHODS

3.1 Study area

Pokhara is sub-metropolitan and second largest city of Nepal. It is the headquarter of both the western development region and the Kaski district. It lies on the geographical coordinates of 28.27° North latitude and 83.97° East longitude. It covers an area of 55.66 sq. km i.e 2.7% area of the district and 0.04% area of the nation. The temperature usually ranges between 2°C to 33°C with an average annual rainfall of 3880 mm whereas the elevation ranges between 827m to 1740m above sea level. There is exclusively great floral and faunal diversity in Pokhara valley due to the prevalence of a wide range of climatic and topographical variations.

Pokhara is not only god gifted place, surrounded by the natural beauty but also rich from the historical view. Here we can find many historical oriented temples reflecting the ancient periods of Nepal which has become the habitat of pigeons too. The pocket areas of present study are:

A. Bhadrakali temple B. Bindhyabasini temple C. Tal Barahi temple

A. Bhadrakali temple

This temple is located at Kudahar, on the top of a small, fine looking hill about 230 feet high and densely covered trees. It is in the east of the city. A pond is situated at base of hill where human settlement is nearby. About 200 *Columba* sp. live here by nesting on the open pen made by temple, roof of temple, nearby trees. The defecation of pigeons are swept in morning and thrown in dustbin. The food mainly comprises barely, wheat, maize, achyataa, remaining of foods, fruits etc. The foods are provided by visitors, temple's organization community and from surrounding resources.

B. Bindhyabasini temple

Bindhyabasini Temple is located at Moharia tole, on a small hill in the North of Pokhara. It is one of the most popular temples, with many Hindus and Buddhist devotees visiting all year round. This temple is near to dense human habitation. Around total of 200 *Columba* sp. comes from nearby forest or lives here nesting on roof of temple. The food mainly comprises barely, wheat, maize, achyataa, remaining of foods, fruits etc provided by visitors, temple's organization community and from surrounding resources. The defecation of pigeons are also swept in morning and thrown in dustbin.

C. Tal Barahi temple

Tal Barahi is one of the most religious and famous pilgrimage sites in Pokhara, devoted to the deity Tal Barahi. About total of 200 *Columba* sp. live here. The temple is located in a small island on the south east section of Fewa lake. The only way of getting to the temple is on boat. The forest and dense tourist area is also nearby. The food mainly comprises barely, wheat, maize, achyataa, remaining of foods, fruits etc. The foods are provided by visitors, temple's organization community and from surrounding resources. They nest on

the open pen made by temple, roof of temple, nearby trees. The defecation of pigeons are swept in morning and thrown in dustbin.

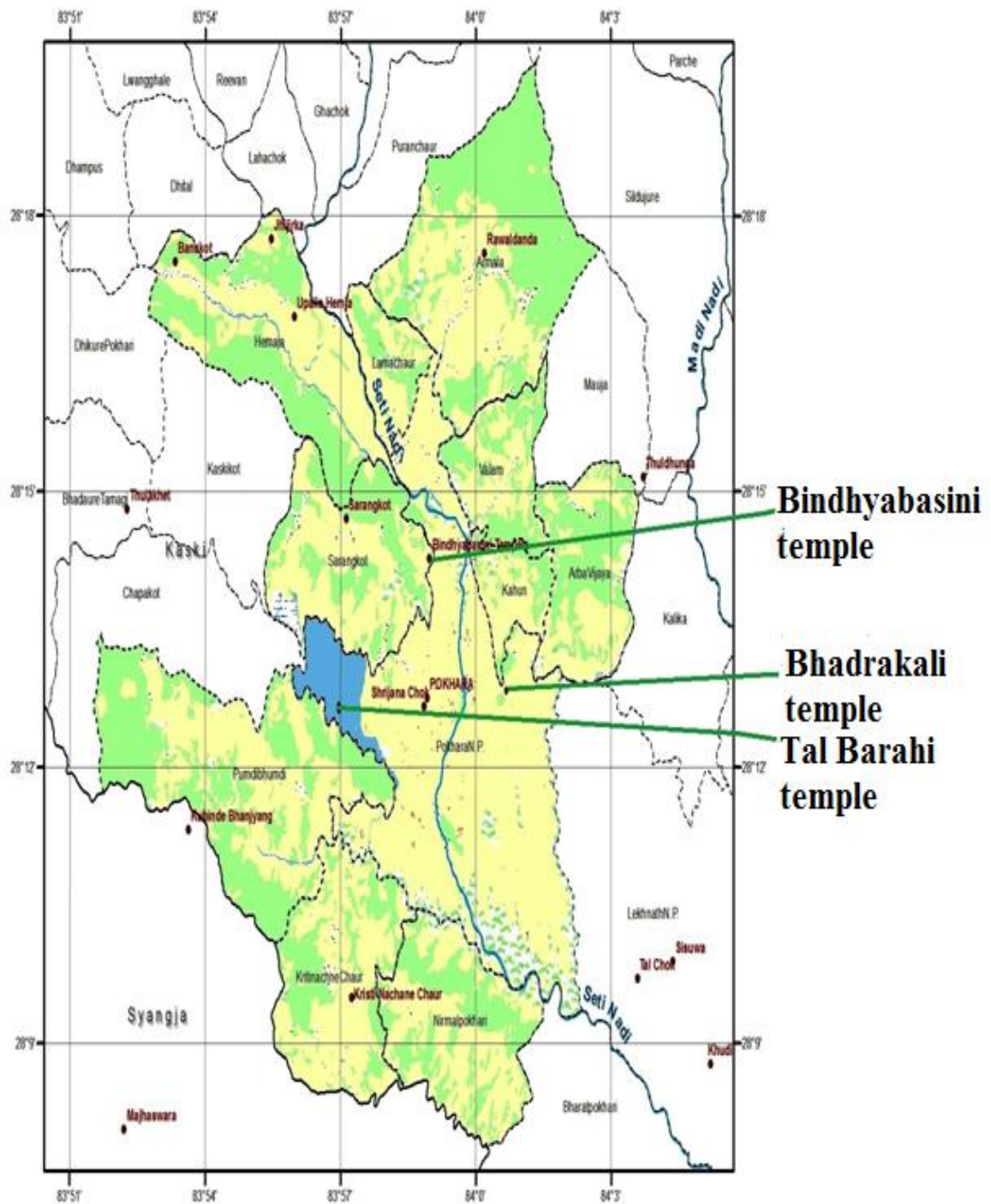


Photo 1: Map of Pokhara valley showing study area (source: LGCDP)

3.2 Materials used

3.2.1 Equipments

- | | |
|------------------------|-----------------------|
| I. Electric microscope | II. Ocular micrometer |
| III. Stage micrometer | IV. Volumetric flask |
| V. Centrifuge machine | VI. Centrifuge tubes |
| VII. Gloves | VIII. Beakers |
| IX. Cover slips | X. Slides |
| XI. Cotton | XII. Tea strainer |
| XIII. Glass rod | XIV. Cavity slide |
| XV. Watch glass | XVI. Rack |
| XVII. Dropper | XVIII. Tooth picks |
| XIX. Mask | XX. Glass vials |
| XXI. Dropper | XXII. Camera |
| XXIII. Gloves | XXIV. Tooth picks |
| XXV. Grains | |

3.2.2 Chemicals

- | | |
|--------------------------------|---------------------|
| I. Potassium dichromate (2.5%) | II. Iodine solution |
| III. Nacl solution | IV. Methylene blue |
| V. Distilled water | |

3.3 Study design

The present study was designed to assess the gastrointestinal parasitic infection in *Columba* sp. of three temples in Pokhara valley. The study comprises:

- a) Selection of temples with abundant *Columba* sp.
- b) Collection of fresh faecal samples in sterile glass vials by opportunistic random sampling.
- c) Preservation of faecal samples in 2.5% of Potassium dichromate solution.
- d) Interview with key informants.
- e) Examination of faecal samples by using iodine wet mount, floatation and sedimentation techniques.
- f) Identification and measurement of eggs and cysts of parasites.

3.3.1 Sample collection method

All the fresh faecal samples of *Columba* sp. were collected by opportunistic random sampling method in early hours of morning. It took each morning for sample collection on each site (three morning). About 5 gm of faecal sample was collected in clean, sterile vial with tooth-pick wearing gloves and mask. It was then preserved with 2.5% potassium dichromate. All the samples collected were labeled properly. The same collection process was repeated for all collected faecal samples.

3.3.2 Preservation of faecal samples

After sample collections, it was preserved in 2.5% Potassium dichromate solution (2.5 gm potassium dichromate powder dissolved in one liter of distilled water). It helps in maintaining morphology of protozoan parasites and preventing further development of helminth eggs and larva.

3.3.3 Sample size

There was around 600 *Columba* sp. in whole study area. Out of these 120 faecal samples (40 each) of *Columba* sp. were collected from the pocket of present study area. It was collected on 16, 17 and 19 March, 2016 A.D. The sample size occupies about 20% of whole population.

3.4 Interview

Verbally administered questionnaires (structured interview) were taken with the key informants of three temples and District Livestock Service Office, Kaski regarding the GI parasites of pigeon.

3.5 Laboratory examination

The collected faecal samples in glass vials were brought from Pokhara to Kathmandu through bus. It was then brought to laboratory of Central Department of Zoology, Kirtipur, Kathmandu for test. The faecal samples were subjected to coprological examination by different concentration technique (floatation and sedimentation) and iodine wet method.

3.5.1 Iodine wet mount

One tooth pick of faecal samples were emulsified in a drop of Lugol's Iodine solution on a clean glass slide and then covered with a clean cover-slip. The smear was examined under electric microscope at 10X and 40X (Soulsby, 1965).

3.5.2 Concentration techniques

Eggs, cysts and trophozoite are often in such low number in faeces, that they are difficult to be detected in direct smears or mounts. Therefore, these procedures were performed which includes floatation and sedimentation techniques (Soulsby, 1982; Zajac and Conboy, 2012).

3.5.2.1 Floatation technique

This technique ensures the eggs float in the floatation liquid, which helps to identify the nematode and cestode eggs as well as protozoan cyst present in *Columba* sp. faeces.

Approximately two gram of faecal samples was put in a beaker and 28 ml of water was added. The sample was grinded lightly with the help of rod or pistle and the solution was filtered by tea strainer. The filtrate solution was poured into a centrifuge tube of 15 ml and centrifuged at 1000 rpm for five minutes. The tube's water was replaced with super saturated Nacl solution and again centrifuged.

After centrifuged, more saturated Nacl solution was added to develop convex meniscus at the top of the tube and one drop of Methylene blue (to stained) was also added. A cover-slip was placed for a five minutes. It was then removed from tube, placed on glass slide and examined microscopically at 10X and 40X. The photographs of eggs and cysts of parasites were taken and identified on the base of shape, shell and size (Soulsby, 1982; Zajac and Conboy, 2012).

3.5.2.2 Sedimentation technique

This technique is used for detection of trematode eggs. It provides a better result as the eggs of trematode are bit heavier than the other. Sediments of centrifuged contents were taken for eggs detection.

Saturated NaCl solution was removed gently from the centrifuge tube after examination of the floatation portion and the sediment content was poured into the watch glass and the content was stirred gently to mix it. One drop of faecal from the mixture was taken to prepare a second slide. The specimen was stained with Iodine wet mount's solution and examined microscopically at 10X and 40X (Soulsby, 1982; Zajac and Conboy, 2012). In this way, two slides were prepared from one sample (one from floatation and one from sedimentation).

3.5.3 Eggs and cysts size measurement

Eggs and cysts size were measured by using micrometry. The calibration factor was found to be 2.588 μm .

3.5.4 Eggs and cysts identification

Table 1: Identification characters of egg and cyst of parasites

S. N.	Parasites	Photo No.	Size (μm)	Shape	Shell	Other features
Helminths						
1.	<i>Ascaridia</i> sp.	9	73-92*45-57	Ovoidal	thick and smooth	
2.	<i>Capillaria</i> sp.	10	53-65*20-35	Barrel shape with bipolar plugs	thick and rough	
3.	<i>Echinostoma</i> sp.	12	88-116*58-69	ellipsoidal	thin	
4.	<i>Heterakis</i> sp.	13	59-75*43-60	ellipsoidal	thick and smooth	
5.	<i>Hymenolepis</i> sp.	14	30-47 diameter	Oncospheres or embryos	thinner and hyaline	
6.	<i>Syngamus</i> sp.	15	78-100*43-60	ellipsoid	thick	4-8 cleavage stage
Protozoa						
7.	Coccidia	11	3-42 diameter	Round or ellipsoidal or ovoid	Usually smooth	Presence of sporocyst

On the basis of size, shape and shell of published literature journals and books (Davis *et al.*, 1971; Soulsby, 1982; Carney, 1991; Ritchie, 1997; Gibbons *et al.*, 2005; Assafa *et al.*, 2006; Schantz, 2006; Cuomo *et al.*, 2009) eggs and cysts were identified (Table. 1:).

3.6 Data analysis

On the basis of laboratory experiment, the data was recorded. The recorded data were coded and entered into Microsoft Excel 2007. Statistical analysis was performed using “R”, version 3.3.1 software packages. Chi-square test was used for statistical analysis of data. In all cases 95% confidence interval (CI) and $P < 0.05$ was considered for statistically significant difference. Percentage was used to calculate prevalence.

4. RESULTS

4.1 General prevalence of GI parasites

Out of 120 faecal samples examined, 83 faecal samples were positive for one or more specific GI parasites, showing 69.16% prevalence of parasitic infection whereas 37 (30.83%) faecal samples were negative.

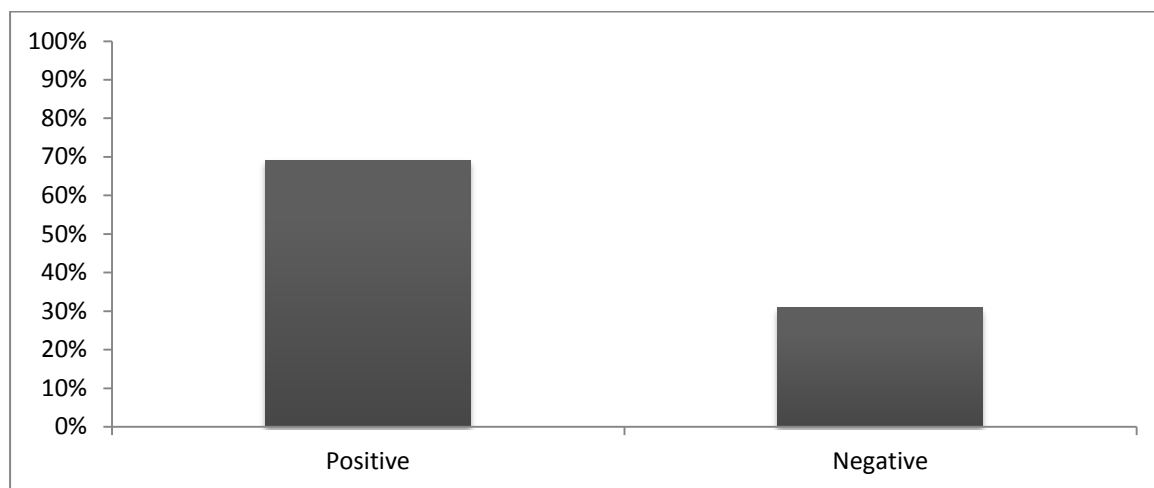


Figure 1: General prevalence of GI parasites

4.2 Prevalence of specific GI parasites

Out of 120 total samples, seven parasites have been identified which are all reported first time in Nepal. The prevalence rate 23 (19.16%) of *Coccidia* and six helminths: *Capillaria* sp. 38 (31.67%) showed highest prevalence, *Ascaridia* sp. 26 (21.66%), *Echinostoma* sp. 9 (7.50%), *Syngamus* sp. 7 (5.83%), *Hymenolepis* sp. 4 (3.33%) and *Heterakis* sp. 3 (2.50%) were recorded.

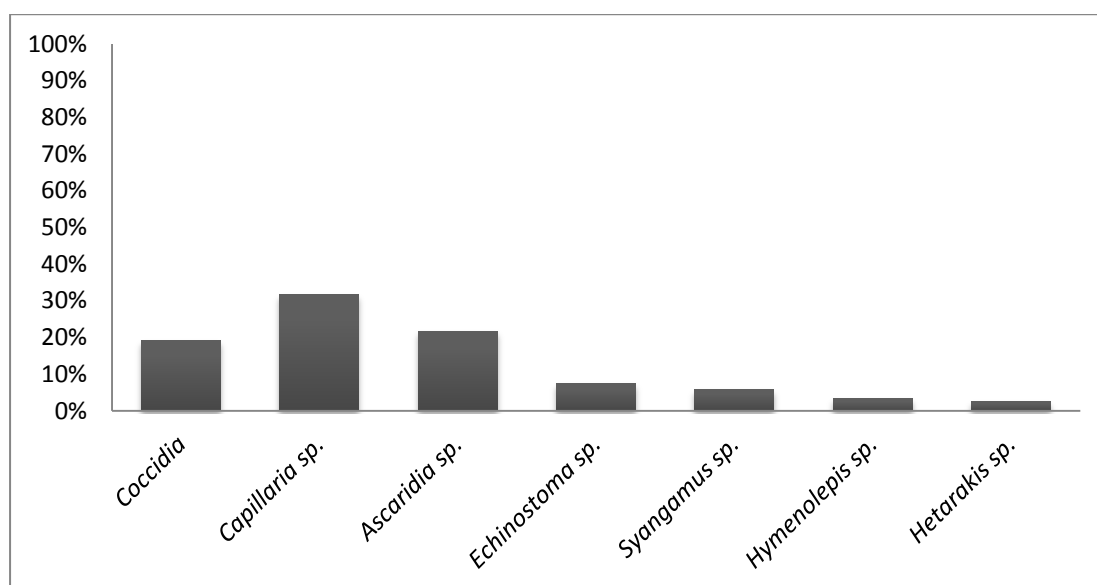


Figure 2: Prevalence of specific GI parasites

4.3 Prevalence of protozoan and helminth parasites

Out of 120 total samples, 66 (55%) were positive with helminths whereas 23 (19.16%) were seen positive with protozoan parasites.

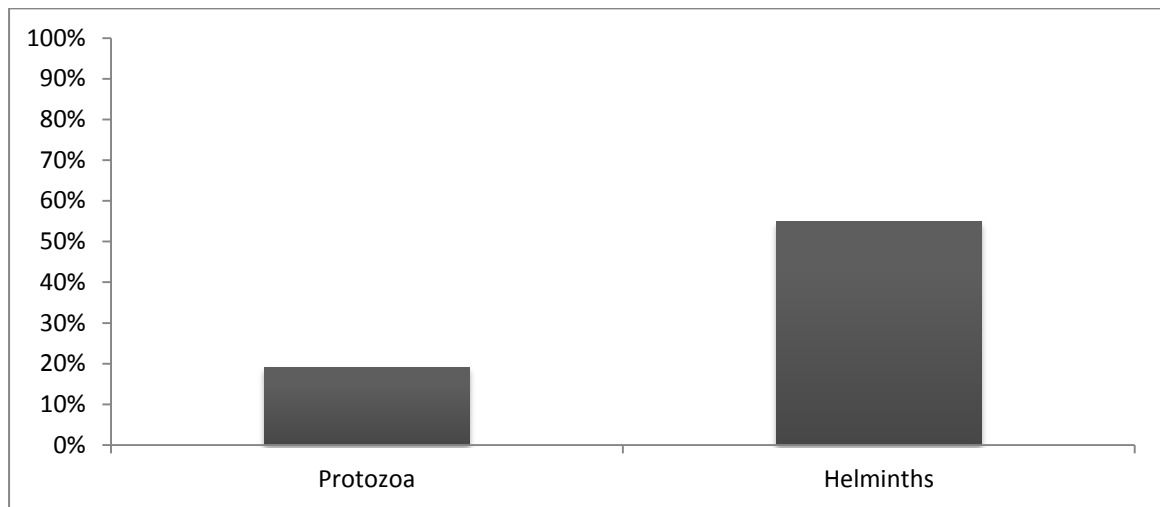


Figure 3: Prevalence of protozoan and helminth parasites

4.4 Area-wise prevalence

Out of three study area, forty samples from each area (Bhadrakali temple, Bindhyabasini temple and Tal Barahi temple) were taken for examination. The area with highest prevalence of GI parasites was in Bhadrakali temple 31 (77.50%) followed by Tal Barahi temple 29 (72.50%) and the lowest was in Bindhyabasini temple 23 (57.50%).

Statistically, the difference in prevalence of GI parasitic infection among study area was found to be insignificant ($\chi^2=0.76328$, $P>0.05$).

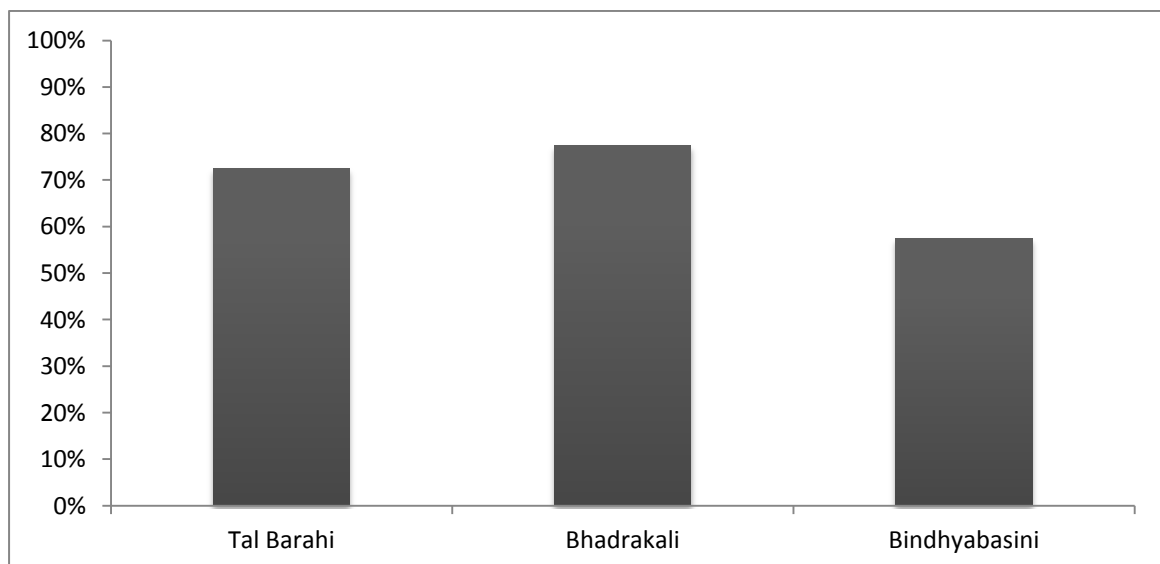


Figure 4: Prevalence of GI parasitic infection among study area

4.5 Infection-wise prevalence

Out of 120 samples, the higher prevalence was of single infection 52 (43.33%) than mixed infections 31 (25.83%).

Statistically, the differences in the prevalence of single and mixed infections were found to be insignificant ($\chi^2 = 3.4728$, $P > 0.05$).

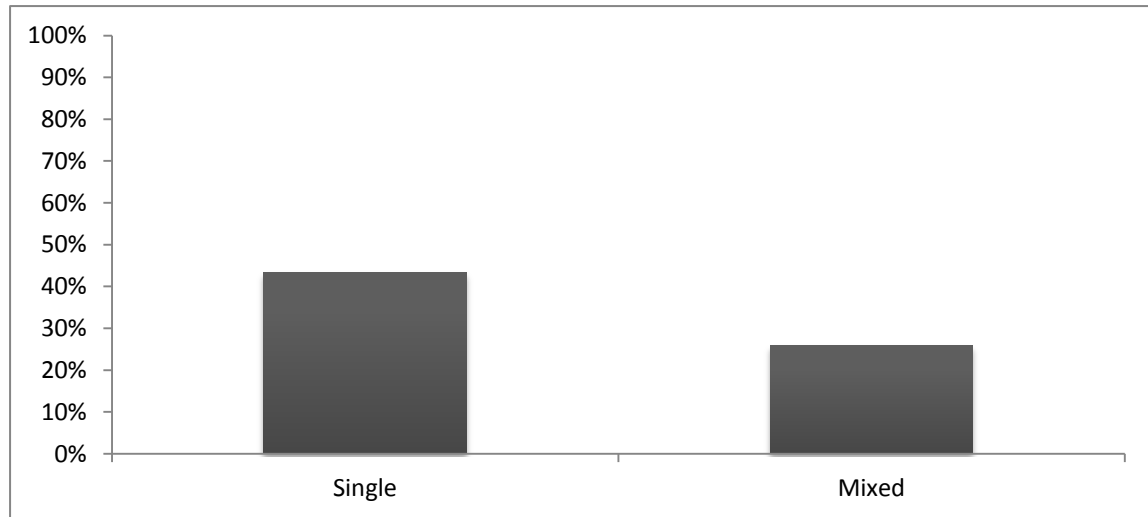


Figure 5: Prevalence of single and mixed infections

4.6 Health care

No any health care such as routinely faecal examination, deworming and other medication regarding the GI parasites of pigeon was found from key informants of three temples and District Livestock Service Office, Kaski.

Sample collection, preservation and laboratory activities



Photo 2: Sample collection at Bindhyabasini temple.



Photo 3: Sample collection at Bhadrakali temple



Photo 4: Sample collection at Tal Barahi temple



Photo 4: Sample preservation



Photo 6: Sample examination (centrifugation)

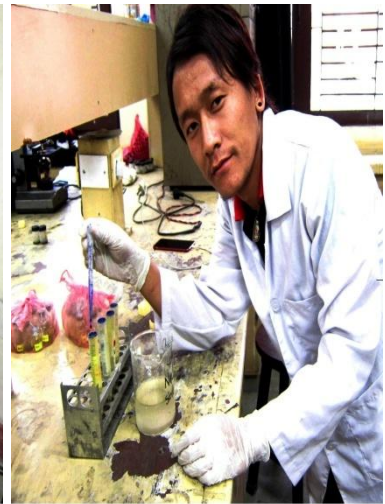


Photo 7: Sample examination (concentration technique)

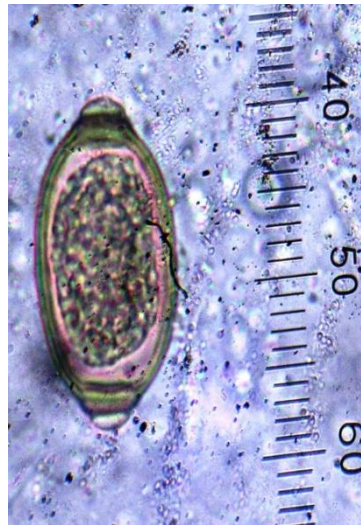


Photo 8: Sample examination (Electric microscope)

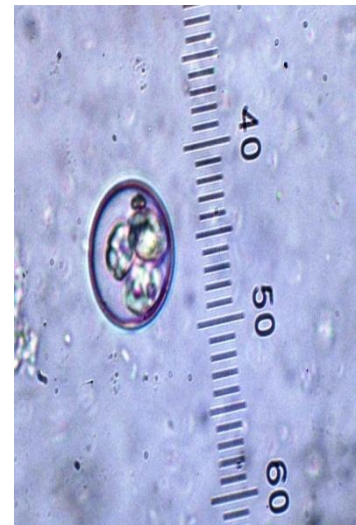
Eggs and cysts of GI parasites in pigeon under 10X*40X electronic microscope



**Photo 9: *Ascaridia* sp. egg
(75.05*56.93)**



**Photo 10: *Capillaria* sp. egg
(53.05*25.88)**



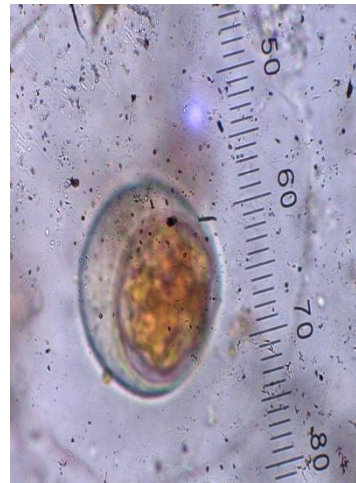
**Photo 11: Coccidia cyst
(20.70 diameter)**



**Photo 12: *Echinostoma* sp. egg
(103.52*62.1)**



**Photo 13: *Heterakis* sp. egg
(46.58*72.46)**



**Photo 14: *Hymenolepis* sp. egg
(46.58 diameter)**



**Photo 15: *Syngamus* sp. egg
(87.99*54.34)**

5. DISCUSSION

Columba sp. are found virtually in every town and city around globe. There are about 100 different breeds and varieties described. They are primarily grain eater and seed eater but also feed on garbage, insects, livestock manure and other food materials.

Generally birds can be affected with both ecto and endoparasites. The major endoparasites reported in pigeons, according to available literatures are *Ascaridia* sp., *Capillaria* sp., *Raillietina* sp., *Eimeria* sp., *Hymenolepis* sp., *Cotugnia* sp. and *Echinostoma* sp.

Three different types of faecal qualitative tests; namely iodine wet mount, floatation and sedimentation techniques were used to determine the prevalence of GI parasites of pigeon in three temples of Pokhara valley and verbally administered questionnaires to find out activities on health care of pigeon regarding the GI parasites.

The general prevalence rate (69.16%) of GI parasites in the present study showed similar prevalence rates 74.14%, 72% and 72.70% obtained by the previous studies (Marques *et al.*, 2007; Sivajothi and Sudhakara, 2015; Gosh *et al.*, 2014) respectively. Three different types of faecal qualitative tests; namely direct smear, floatation and sedimentation techniques that were used in present study was also used by them.

The general prevalence rate of present study was lower as compared to 100% prevalence rate of others finding (Al-Barwari and Saeed, 2012). The 100% prevalence rate of previous study (Al-Barwari and Saeed, 2012) was because of combine prevalence rate of fungi, protozoa, cestodes, nematodes and arthropods. Specific prevalence rate of GI parasites was not given. Hundred percent parasitic infections may be due to infection or re-infection (directly or indirectly) of parasites which indicates poor management and control efforts in the birds or in the immediate environment (Opara *et al.*, 2012).

The general prevalence rate of present GI parasites was higher than 53.57%, 46.70% and 46.12% obtained by previous studies (Patel *et al.*, 2000; Sari *et al.*, 2008; Opara *et al.*, 2012) respectively. Although this rate of prevalence also indicates, health of pigeon is in risk. Patel *et al.* (2000) has studied upon captive birds of Gujarat zoos. Since there is more care in zoos as compared to outside, so this might be the main reason of less prevalence than present study and only sedimentation technique was used by them. Opara *et al.* (2012) examined only adult birds of street pigeons (*Columba livia*) and accepted certain level of host immunological response. They have considered moderate prevalence may be attributed to the food searching habits of the pigeons of not scratching below the surface soil where most infective stages of these nematodes are hidden. Sari *et al.* (2008) has studied parasites of domestic (*Columba livia domestica*) and wild (*Columba livia livia*) pigeons together in Nigde, Turkey. Combination study of wild and domestic pigeons as well as different seasonal and geographical variation might be cause of low prevalence rate of parasites than present study.

Among seven different GI parasites identified in present study, the prevalence rates of *Capillaria* sp. (31.67%) were higher. The prevalence rate of *Capillaria* sp. (31.67%) were similar with 24.20% and 22% of previous studies (Sari *et al.*, 2008; Gosh *et al.*, 2014) respectively whereas it was higher than 17.40%, 14.14%, 13.20% and 5.55% as compared

to others findings (Sivajothi and Sudhakara, 2015; Bahrami *et al.*, 2013; Patel *et al.*, 2000; Borji *et al.*, 2012) respectively. Some *Capillaria* sp. (*Capillaria anatis*, *Capillaria obsignata*) have a direct life cycle. Domestic and wild birds ingest these infective eggs with contaminated food or water whereas other species (*Capillaria annulata*, *Capillaria bursata*, *Capillaria caudinflata*) have various earthworm sp. as obligate intermediate hosts. The larva develops inside the earthworms which become infective in two to four weeks and can survive for years inside it (Davis *et al.*, 1971). Tal Barahi temple is surrounded by Fewa lake and Bhadrakali temple is near to pond. So there is high and equal prevalence of *Capillaria* sp. in both study area than Bindhyabasini temple. The high prevalence of *Capillaria* sp. in present study than other author might be due to contaminant drinking resources, food, soil and difference in geographical area.

In present study, prevalence rate (21.66%) of *Ascaridia* sp. were consistence with 30%, 28.33%, 20.75%, 20.37%, 16.66%, 15.50% and 15.21% obtained by previous studies (Mushi *et al.*, 2000; Begum and Sehrin, 2012; Patel *et al.*, 2000; Borji *et al.*, 2012; Radfar *et al.*, 2011; Msoffe *et al.*, 2010; Radfar *et al.*, 2012) respectively. The prevalence of present study was higher as compared to 5.10%, 4.04% and 1.20% of previous studies (Sari *et al.*, 2008; Bahrami *et al.*, 2013; Natala *et al.*, 2009) respectively. This is probably due to different climatic factors in the study areas (Chege *et al.*, 2015). The possible migration of *Ascaridia* sp. to liver, trachea and lung for development also suggest low prevalence (Michel, 1974). The prevalence rate of present study was lower as compared to 35% and 33.33% reported in previous studies (Gosh *et al.*, 2014; Sivajothi and Sudhakara, 2015) respectively. It might be due to difference in sample collection method. As they have collected samples from adult, squab and nestling pigeons.

The prevalence rate (2.50%) of *Heterakis* sp. were similar to 9.02%, 3.7% and 3.3% obtained by previous studies (Gosh *et al.*, 2014; Sari *et al.*, 2008; Adang *et al.*, 2008) respectively. This species are seen less in winter season in temperate region (Permin and Jorgen, 1998). *Heterakis gallinarum* is non-pathogenic, but a vector for *Histomonas meleagridis* which is highly pathogenic etiologic agent of “Black-head” disease lethal to chickens, turkeys, pheasants and other fowls (Cheng, 1973).

Moreover, the prevalence rate (5.83%) of *Syngamus* sp. infection of the present study were similar with 9.09%, 3% and 1.70% of previous studies (Bahrami *et al.*, 2013; Gosh *et al.*, 2014; Sari *et al.*, 2008) respectively.

The prevalence rate (3.33%) of *Hymenolepis* sp. were similar to 13.30% and 3.70% obtained by previous studies (Adang *et al.*, 2009; Sari *et al.*, 2008) respectively. It was also recorded in other studies (Adang *et al.*, 2008; Olsen and Braun, 1980). Begum and Sehrin (2012) found higher prevalence rate (63.33%) of *Hymenolepis* sp. than present study. Since they have took a year for sample collection, sample size of 60 which is half of present study and examined pigeon through separated alimentary canal might cause high difference between our studies. As well as the geographical area is different and the pigeons of temples are generally fed grains by the people which reduce possible threats from intermediate host might be another reason of low prevalence of *Hymenolepis* sp. in present study.

Echinostoma sp. was found with 7.50% of prevalence rate in present study. It is lower than prevalence rates of 25% and 20% as compared to others finding (Musa *et al.*, 2011;

Begum and Sehrin, 2012) respectively. *Echinostoma* sp. has three hosts in their life cycle: first intermediate host, second intermediate host and a definitive host. Snail species such as *Lymnaea* sp. are common intermediate hosts (Huffman and Fried, 1990). Ledwoń *et al.* (2016) diagnosed only two known cases of fluke invasions in racing pigeons (*Columba livia*) originating from different regions of Poland over four years. The lower prevalence of *Echinostoma* sp. might be due to need of more than two intermediate host, favorable environment as well as plenty availability of grains and seeds as food for pigeons in present study area.

The prevalence rate (19.16%) of coccidian parasites in present study showed similarity with 17.92%, 11% and 7.07% of previous studies (Patel *et al.*, 2000; Gosh *et al.*, 2014; Bahrami *et al.*, 2013) respectively. This rate was lower as compared to 49.20%, 46.27%, 46.20%, 43.15% and 31% of previous studies (Natala *et al.*, 2009; Nagwa *et al.*, 2013; Sari *et al.*, 2008; Radfar *et al.*, 2011; Sivajothi and Sudhakara, 2015) respectively. Coccidiosis infected birds generally exhibit loss of appetite, weakness, ruffled feathers, bloody diarrhea and can only be diagnosed by post-mortem examination (Dingle and Shanawany, 1999). The difference in prevalence rates of coccidian might be due to difference in practice of management area, hygiene of pens, flock structure, samples collected and laboratory techniques. In the temperate regions, the eggs of *Eimeria* cannot embryonate and develop to infectivity during the winter season of temperature below 10-15°C (Permin and Jorgen, 1998) might also cause low prevalence of Coccidia.

The present of *Raillietina* sp. have been recorded in different literature during literature review. In present study, there is absent of *Raillietina* sp. which is also absent in study of (Bahrami *et al.*, 2013).

In Nepal, *Raillietina torquata* was recorded by (Meggett, 1924) whereas (Sharma, 1943) recorded *Raillietina kantipura*, *Raillietina nagpurensis* and *Raillietina nripendra* using dissection method from Kathmandu. *Raillietina* sp. requires two different host for complete life cycle. The definitive hosts are mostly wild and domestic birds, and sometimes human and several insects such as ants and beetles as intermediate hosts. Diagnosis of *Raillietina* sp. is usually done by post-mortem upon autopsy, since proglottids are seen in faeces rather than eggs (Ritchie *et al.*, 1997) might be cause of absence of *Raillietina* sp. or might be because of plenty availability of grains and seeds in study area that prevents the intermediate host of *Raillietina* sp. as food.

The prevalence of helminths were higher than protozoan parasites in present study. Higher prevalence of helminths than protozoan parasites was also reported previously (Patel *et al.*, 2000). The prevalence rate (55.50%) of helminths was similar with prevalence rates of 56.10%, 48.30% and 42.15% obtained by previous studies (Adang *et al.*, 2009; Adang *et al.*, 2008; Radfar *et al.*, 2011) respectively.

The prevalence rates of helminths 36.90%, 33.33%, 23.50%, 18.70% and 12.50% shown by previous studies (Borji *et al.*, 2012; Musa *et al.*, 2011; Dranzoa *et al.*, 1999; Natala *et al.*, 2009; Olsen and Braun, 1980) respectively were lower than present study whereas prevalence rates of 100%, 84.78% 79.50% and 79.20% obtained by previous studies (Begum and Sehrin, 2012; Radfar *et al.*, 2012; Msoffe *et al.*, 2010; Bahrami *et al.*, 2013) respectively were higher than present study. Among protozoan, 19.16% prevalence rates was recorded in present study which was lower than 72%, 46.27% and 43.13% of

previous studies (Natala *et al.*, 2009; Nagwa *et al.*, 2013; Radfar *et al.*, 2011) respectively. The overall prevalence of various parasites differs greatly among the previous reports as well as when compared with present observation. This might be due to variance in sample collection methods, sample size and sample examination methods. Diversity of bird endoparasite assemblages may be related with many factors, which may include home range, behaviour, size and roosting habit of the host. This may also be attributed to difference in the geographical areas and period of study (Begum and Sehrin, 2012).

The presence of dense forest, pond, canal and human habitation might be the cause of high prevalence rate of GI parasites in Bhadrakali temple than other pocket area. Statistically, difference in prevalence of GI parasitic infection among study area was found to be insignificant ($\chi^2=0.76328$, $P>0.05$). It might be because of similar climate, food resources and environment. The low prevalence rates of protozoan parasites than helminths parasites may be because of pigeons have high antibody titres, to the protozoan parasite (Mushi *et al.*, 2000). The high prevalence of helminth infections recorded in this study could be an indication of a high incidence of the infective stages and intermediate hosts of the parasites in places where these pigeons are reared. The intermediate hosts of these parasites; beetles, pill bugs, ants, earthworms and snails which form part of the diet of Pigeons (Adang, 1999).

There was no significant differences ($\chi^2=3.4728$, $P>0.05$) in the prevalence of single and mixed infections in present study. The higher prevalence of single infection (43.33%) was seen than mixed infections (25.83%). The high prevalence of single infection was also seen in the previous studies (Adang *et al.*, 2008; Adang *et al.*, 2009; Bahrami *et al.*, 2013). The high prevalence of single infections in the pigeons may suggest a form of competition that kept the other species away (Kennedy, 1975). It may be also because of crowding effect in pens and nest.

No any activities on health care of pigeon such as routinely faecal examination, deworming and other medication regarding the GI parasites was found during verbally administered questionnaires taken with the priest of Bindhyabasini temple, worker of Tal Barahi temple and management chief of Bhadrakali temple. The same answer was found from District Livestock Service Office, Kaski too. This confirmed the high susceptibility of GI parasites in pigeons.

This is the first study on GI parasites of pigeons in three temples of Pokhara valley and reported one subclass of protozoan: *Coccidia* 23 (19.16%) and six genera of helminths: *Capillaria* sp. 38 (31.67%), *Ascaridia* sp. 26 (21.66%), *Echinostoma* sp. 9 (7.50%), *Syangamus* sp. 7 (5.83%), *Hymenolepis* sp. 4 (3.33%) and *Hetarakis* sp. 3 (2.50%) which are first time in Nepal as their presence were not found in research articles.

GI parasites can be controlled through effective management practices, daily cleaning of cages and surrounding, washing dishes, quarantine new birds before entrance to the flock, clean water resources, eradication of intermediate host, regular treatments of anthelmintic, anticoccidials drugs and dusting of birds with pesticides as well as educating the breeders of birds, visitors in temples.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The general prevalence of GI parasites of pigeons (*Columba* sp.) was found to be 69.16% with higher prevalence rates of *Capillaria* sp. (31.67%). Total of seven GI parasites that includes one subclass of protozoan: *Coccidia* 23 (19.16%) and six genera of helminths: *Capillaria* sp. 38 (31.67%), *Ascaridia* sp. 26 (21.66%), *Echinostoma* sp. 9 (7.50%), *Syangamus* sp. 7 (5.83%), *Hymenolepis* sp. 4 (3.33%) and *Heterakis* sp. 3 (2.50%) were identified and reported first time in Nepal. The higher prevalence of GI parasites was in Bhadrakali temple 31 (77.50%) followed by Tal Barahi temple 29 (72.50%) and the lowest was in Bindhyabasini temple 23 (57.50%). Statistically, the difference in prevalence of GI parasitic infection among study area was found to be insignificant ($\chi^2=0.76328$, $P>0.05$). The prevalence of helminths 66 (55%) were higher than protozoan parasites 23 (19.16%) whereas the difference in prevalence of single infection 52 (43.33%) and mixed infections 31 (25.83%) were insignificant ($\chi^2 =3.4728$, $P>0.05$). No any activities on health care of pigeon regarding the GI parasites were found.

This is the first study on GI parasites of pigeons in three temples of Pokhara valley. The study indicated that pigeons in three temples of Pokhara valley were highly susceptible to GI parasites. Therefore sustainable ways for controlling the parasitic infection and further studies need to be designed for the health and conservation of pigeons.

6.2 Recommendations

- ❖ Health care programmes of pigeon such as routinely faecal examination and deworming should be done by its concerns for effective control of GI parasites.
- ❖ Further identification on species level of parasites could be done.
- ❖ Seasonal-wise study can be studied.

7. REFERENCES

- Adang, K. L. 1999. Some aspect of the biology of four columbid species in Zaria, Nigeria. M.sc. Thesis, Ahmadu Bello University.
- Adang, K. L., Oniye, S. J., Ajanusi, O.J., Ezealor, A.U and Abdu, P.A. 2008. Gastrointestinal helminth of the domestic pigeons (*Columba livia domestica* Gmelin, 1789 Aves:Columbidae) in Zaria, Northern Nigeria. Science World Journal, **3**: 33-7.
- Adang, K. L., Oniye, S. J., Ezealor, A. U., Abdu, P. A., Ajanusi, O. J. and Yoriyo, K. P. 2009. Ectoparasites and intestinal helminthes of Speckled Pigeon (*Columba guinea*) Hartlaub and Finsch, 1870) in Zaria, Nigeria. Science World Journal, **4**(2): 1-5.
- Al-Barwari, S. and Saeed, I. 2012. The parasitic communities of rock pigeon (*Columba livia*) from Iraq: component and importance. Turkiye Parazitol Derg, **36**: 232.
- Alves, M.G., Fernandes, G., Silva, T., Lopes, R. and Andreatti, R. 2008. Intestinal protozoan parasites with zoonotic potential in birds. Parasitol Res **103**: 1237–1240.
- Assafa, D., Kibru, E., Nagesh, S., Gebreselassie, S., Deribe, F. and Ali, J. 2006. Medical parasitology. Ethiopia Public Health Training Initiative
- Bahrami, A.M., HosseinI, E. and Razmjo, R. 2013. Important parasite in pigeon, its hematological parameter and pathology of intestine. World Applied Sciences Journal, **21**(9): 1361-1365.
- BCN and DNPWC 2016. Birds of Nepal: An official checklist, Kathmandu, Nepal.
- Begum, A. and Sehrin, S. 2012. Gastrointestinal helminths in pigeon (*Columba livia* Gmelin,1789). J. Asiat. Soc. Bangladesh, Sci., **38**(1): 93-98.
- Borji, H., Moghaddas, E., Razmi, R.R. and Azad, M. 2012. A survey of ecto and endo parasites of domestic pigeons (*Columba livia*) in Mashhad, Iran. Iranian Journal of Veterinary Science and Technology, **4**(2): 37-42.
- Burger, R.E. 1974. Ringneck Doves American cage-bird magazine, 25 pp.
- Carney, W.P. 1991. Echinostomiasis - a snail-borne intestinal trematode zoonosis. Southeast Asian Journal of Tropical Medicine and Public Health. **22**: 206–211.
- Chege, H.W., Kemboi, D.C., Bebora, L.C., Maingi, N., Mbuthia, P.G., Nyaga, P.N, Njagi., L.W. and Githinji, J. 2015. Studies on seasonal prevalence of ecto- and endo parasites in indigenous chicken of Mbeere Subcounty, Kenya.
- Cheng, T. 1973. General parasitology. Academic Press, New York, San-Francisco and London
- Clarkson, T.B., Prichard, R.W., Lofland, H.B. and Goodman, H.O. 1963. The pigeon as a laboratory animal. Lab. Anim. Care, **13**: 767.
- Cuomo, M.J., Noel, L.B. and White, D.B. 2009. Diagnosing medical parasites: a public health officers guide to assisting laboratory and medical officers. Air education and training command Randolph AFB Texas.
- Davis, J.W., Anderson, R.C., Karstad, L. and Trainer, D.O. 1971. Infectious and parasitic diseases of wild birds. Iowa State University Press, USA, 185-233 pp.

- Dingle, J. and Shanawany, M. 1999. Ostrich production system. FAO Animal Production and Health Paper 144 1st edition. FAO, Rome, 256 pp.
- Dranzoa, C., Ocaido, M & Katete, P. 1999. The ecto-, gastro-intestinal and haemo parasites of live pigeons (*Columba livia*) in Kampala, Uganda. Avian Pathology, **28**: 119-124.
- Galloway, H.J. 1972. Farm animal health and disease control. Longman, London.
- Ghosh, K.K., Islam, M.S., Sikder, S., Das, S., Chowdhury, S. and Alim, M.A. 2014. Ecto and gastrointestinal parasitic infections of pigeon at Chittagong Metropolitan area, Bangladesh. The Journal of Advances in Parasitology, **1**(1): 9–11.
- Gibbons, L.M., Jacobs, D.E., Fox, M.T. and Hansen, J. 2005. The RVC/FAO guide to veterinary diagnostic parasitology: faecal examination of farm animals for helminth parasites.
- Haag-Wackernagel, D. 2006. Human diseases caused by feral pigeons. Advances in Vertebrate Pest Management. **4**: 31–58.
- Haag-Wackernagel, D. and Bircher, A. J. 2009. Ectoparasites from feral pigeons affecting humans. Dermatology, **220**(1): 89-92
- Hollander, W.F. 1954. Pigeons in research. Proc. Animal Care Panel, **5**: 71.
- Kendall, R.J. and Scanlon, P.F. 1981. Propagation of a laboratory ringed turtle dove colony. Poult Sci, **60**: 2728.
- Kennedy, C. R. 1975. Ecological animal parasitology. Blackwell scientific publications, Oxford, London, Edinburgh, Melbourne.
- Ledwoń, A., Dolka, B., Piasecki, T., Dolka, I and Szeleszczuk, P. 2016. Invasion of flukes of the Echinostomatidae Family in racing pigeon (*Columba livia* var. *domestica*) lofts. American Association of Avian Pathologists, Avian Diseases, **60**(2): 523-527.
- Levi, W.M. 1969. The Pigeon. Levi Publishing, Sumter SC.
- Magwisha, H., Kassuku, A., Kyvsgaard, N. and Permin, A. 2002. A comparison of the prevalence and burdens of helminth infections in growers and adult free range chickens. Tropical Animal Health Production, **34**(3): 205-214.
- Lindström, K.M. 2000. Bird-parasite interactions: Using Sindbis virus as a model system.
- Marques, S.M., Quadros, R.M, Da-Silva, C.J. and Baldo, M. 2007. Parasites of pigeons (*Columba livia*) in urban areas of langes, Southern Brazil, Journal of Comunicaciones *Parasitol Latinoam.* **62**: 183-187.
- Matur, B. and Dawam, N.M.Y. 2010. Gastrointestinal helminth parasites of local and exotic chickens slaughtered in Gwagwalada, Abuja (FCT), Nigeria. New York Science Journal, **3**(5): 96-99.
- Meggitt, F.J. 1924. Some avian cestodes from Nepal. Bull. Brt. Mus. Vol 12.
- Michel, J.F. 1974. Arrested development of nematodes and some related phenomena in advances in parasitology. In: Ben Dawes (Ed) publ: Academic Press London and New York, 277-366 pp.
- Msoffe P.L.M., Muhairwa, A.P., Chiwanga G.H. and Kassuku, A.A. 2009. A study of ecto- and endo-parasites of domestic pigeons in Morogoro Municipality. Tanzania. African Journal of Agricultural Research **5**(3): 264-2672.

- Mumcuoglu, K.Y., Banet-Noach, C., Malkinson, M., Shalom, U. and Galun, R. 2005. Argasid ticks as possible vectors of West Nile virus in Israel. *Vector-Borne & Zoonotic Diseases*, **5**(1):65-71.
- Musa, S., Afroz, S.D. and Khanum, H. 2011. Occurrence of ecto- and endo parasites in pigeon (*Columba livia*). *Rajshahi University Zoological Society*, **30**: 73-75.
- Mushi, E.Z., Binta, M.G., Chabo, R.G., Ndebele, R. and Panzirah, R. 2000. Parasites of Domestic pigeons (*Columba livia domestica*) in Sebele, Gaborone, Botswana. *Journal of the South African Veterinary Association, Botswana College of Agriculture, Private Bag* **71**(4): 249–250.
- Nagwa, E.A., El-Akabawy, L.M., El-Madawy, R.S. and Toulan, E.I. 2013. Studies on intestinal protozoa of poultry in Gharbia governorate. *Benha Vet. Med. J*, **25**(2):78-83.
- Natala, A.J., Asemadahun, N.D., Okubanjo, O.O., Ulayi, B.M., Owolabi, Y.H., Jato, I.D. and Yusuf, K.H. 2009. A survey of parasites of domesticated pigeon (*Columba livia domestica*) in Zaria, Nigeria. *International Journal of Soft Computing*, **4**(4): 148-150.
- Opara, M.N., Ogbuewua, I.P., Iwujia, C.T., Njokua, L., Ihesiea, E.K. and Etuka, I.F. 2012. Blood characteristics, microbial and gastrointestinal parasites of street pigeons (*Columba livia*) in Owerri Imo State. *Nigeria Scientific Journal of Animal Science*, **1**(1): 14-21.
- Olsen, O.W. and Braun, C.E. 1980. Helminth parasites of Band-tailed Pigeons in Colorado. *Journal of Wildlife Diseases*, **16**(1): 65-66.
- Patel, P.V., Patel, A.I., Sahu, R.K. and Vyas, R. 2000. Prevalence of gastro-intestinal parasites in captive birds in Gujarat zoos. *Zoos Print Journal*, **15**(7): 295-296.
- Permin, A. and Jorgen W.H. 1998. *Epidemiology, diagnosis and control of poultry parasites*. FAO, Roma (Italia).
- Radfar, M.H., Fathi, S., Asl, E.N., Dehaghi, M.M. and Seghinsara, H.R. 2011. A survey of parasite of domestic pigeons (*Columba livia domestica*) in South Khorasan, Iran, *Veterinary Research*, **4**(1): 18-23.
- Radfar, M.H., Khedri, J., Adinehbeigi, K., Nabavi, R and Rahmani, K. 2012. Prevalence of parasites and associated risk factors in domestic pigeons (*Columba livia domestica*) and free-range backyard chickens of Sistan region, east of Iran. *J Parasit Dis*, **36**(2): 220–225.
- Ritchie, B.W., Hsarrison, G.J., Zantop, D. and Harrison, L.R. 1997. *Avian medicine: principles and application, abridged edition*. Idaho Falls, ID: Wingers Publishing, 1007-1028 pp.
- Sari, B., Karatepe, B., Karatepe, M. & Kara, M. 2008. Parasites of domestic pigeon (*Columba livia domestica*) and wild pigeons (*Columba livia livia*) in Niğde, Turkey. *Bull Vet Inst Pulawy*, **52**: 551-554.
- Schantz, P.M. (2006). Tapeworms (Cestodiasis). *Gastroenterology Clinics of North America*, **25**(3): 637-653.
- Sharma, K.N. 1943. Notes on cestodes collected in Nepal. *Indian vet. Journal*, **20**(2): 53-57.

- Sivajothi, S. and Sudhakara, R.B. 2015. A study on the gastro intestinal parasites of domestic pigeons in YSR Kadapa district in Andhra Pradesh, India. *Journal of Dairy, Veterinary & Animal Research*, 2(6): 57.
- Solusby, E.J.L, 1965. Textbook of veterinary clinical parasitology. Volume I. Helminths.
- Soulsby, E.J.L. 1982. Helminths, arthropods and protozoa of domesticated animals 7th ed. Bailliere Tindall, London, 809 pp.
- Sturtevant, J. and Hollander, W.F. 1978. Breeding pigeons at the laboratory. *Pigeon Science and Genetics Newsletter* 8 (suppl.), 7 pp.
- Williams, D.E. and Corrigan, R.M. 1994. The handbook: prevention and control of wildlife damage. University of Nebraska, Lincoln, 69 pp.
- Weber, W. 1979. Health hazards from pigeons, starlings and english sparrows. Thomson Publications, Fresno, California. 137 pp.
- Zajac, A.M. and Conboy, G.A. 2012. Veterinary clinical parasitology. Eighth edition. American Association of Veterinary Parasitologist. Blackwell publishing, Oxford, U.K.
- Zwart, P. 1986. Pigeons and doves. In Fowler M E (ed.) *Zoo & wild animal medicine* 2nd ed. WB Saunders, Philadelphia, 440–445 pp.
- LGCDP, 2013. Local governance and community development programme. http://lgcdp.gov.np/sites/default/files/GIS/44_Pokhara.jpg. Accessed on 11 August, 2016.