GASTRO-INTESTINAL PARASITES IN EXOTIC AVIAN FAUNA OF CENTRAL ZOO, JAWALAKHEL, LALITPUR, NEPAL

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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 source of information have been specifically acknowledged by reference to the author (s) or institution (s).

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RECOMMENDATION AND LETTER OF APPROVAL

This is recommended that the thesis entitled "GASTRO-INTESTINAL PARASITES IN EXOTIC AVIAN FAUNA OF CENTRAL ZOO, JAWALAKHEL, LALITPUR, NEPAL" has been carried out by Ashok Bohara 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. Subsequently, the aforementioned thesis is approved for the examination and submitted to the Tribhuvan University in the partial fulfillment of Master's Degree of Science in Zoology with special paper Parasitology.

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Ashok Bohara entitled "GASTRO-INTESTINAL PARASITES IN EXOTIC AVIAN FAUNA OF CENTRAL ZOO, JAWALAKHEL, LALITPUR, NEPAL" has been accepted as a partial fulfillment of Master's Degree of Science in Zoology with special paper Parasitology.

EVALUATION COMMITTEE

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LIST OF ABBREVIATIONS

Abbreviated form	Details of abbreviations
μm	- Micrometer
CDZ	- Central Department of Zoology
CI	- Confidence Interval
EPG	- Egg per gram
GI	- Gastro-Intestinal
NaCl	- Sodium Chloride
NTNC	- National Trust for Nature Conservation
rpm	- Round per minute

ABSTRACT

The present study was conducted to determine the general prevalence, helminthes and protozoan wise prevalence of gastrointestinal parasites and assess the management practices of 16 species of exotic avian fauna present at Central Zoo, Jawalakhel, Lalitpur, Nepal. Altogether 100 faecal samples were collected on third week of April, 2017. The qualitative examination of faecal samples were done by iodine wet mount for protozoan and floatation and sedimentation technique for helminthes whereas Microsoft Excel 2013 and "R" version 3.3.1 software package was used for data analysis. Out of total 100 faecal samples examined, 54 fecal samples were positive with overall prevalence of 54%. Total of five species of gastrointestinal parasites were identified that include one genera of protozoa: *Eimeria* sp. (37 %) and four genera of helminthes: *Ascaridia* sp. (23 %) showed the highest prevalence followed by *Trichostrongylus* sp. (10 %), *Strongyloides* sp. (7 %) and *Codiostomum* sp. (3 %). Although the prevalence rate of protozoan parasites (37 %) was higher than prevalence rate of helminthes parasites (33 %) but statistically the difference in prevalence of helminth and protozoan parasites was found to be insignificant ($p = 0.656$, $\chi^2 = 0.197$). Single infection was found to be higher than mixed infection. Statistically, the difference in prevalence of single and mixed infection was found to be significant ($p = 0.034$, $\chi^2 = 4.481$). Among mixed infections, double infection showed the highest infection rate (76.19 %) than multiple infection (23.80 %). Although the study indicates that exotic avain fauna at Central Zoo were infected to gastrointestinal parasites but the maintenance of hygiene and deworming programme is found to be effective because the birds kept inside the cage are active and death rate is very low but the possibility of reappearance of parasitic infection is always there due to stress and also due to closer proximity with other foraging birds that directly transmit parasites.

1. INTRODUCTION

1.1 Background

Zoological garden (Zoo) is an ex-situ form of conservation where wild animals are keep alive in cages for exhibition, education or research and protection of endangered species (Thawait *et al*., 2014). Zoo plays a vital role to educate the public regarding wildlife and their habitats. The zoo has been partially successful in increasing the population of endangered species using its captive breeding facilities (Mir *et al.,* 2016). Avian fauna are kept in zoological garden during a part or their entire life in the captive form (Lima *et al.,* 2016). Birds are the integral part of every ecosystem that plays important role for a wide range of purposes including fundamental research, aesthetic, applied veterinary medical studies and toxicology (Otegbade and Morenikeji, 2013). Zoo aggregates many number of animal species including birds in a close relation and some of these animals are exotic to the geographical location of the ecological garden. Repopulation of endangered species and conservation of wild animals and birds in zoological garden are management strategies (Ajibade *et al.*, 2010). Zoo provide opportunity for researcher to study species that are difficult to observe in their natural habitat. Helminth diseases are an important problem for captive birds and animals in Zoological garden. Therefore, a comprehensive knowledge of parasitic disease is necessary, especially when animals are breed in captivity and then release back to the wild (Gurler *et al*., 2010).

1.1.1 Central Zoo of Nepal

Central Zoo is the only one Zoo in Nepal which was establish in 1932 A.D. by the late Prime Minister Juddha Sumsher JBR. It is located at Jawalakhel, Lalitpur with an area of 16 hectors. The area of zoo is not sufficient enough for its inhabitants to have an ideal captive environment. On 29th December 1995, the management of Central Zoo was formally handed over to the National Trust for Nature Conservation. Central Zoo gives priority for the preservation of endangered species of Nepal like One Horned Rhinoceros, Royal Bengal Tiger, Gharial Crocodile, Golden Monitor Lizard, Crimson Horned Pheasant etc. The Zoo has performed animal exchange program with some zoo of abroad. At present, there are 830 wild animals belonging to 103 species. Among these there are 34 species of 262 mammals, 46 species of 377 birds, 11 species of 35 reptiles and 12 species of 156 fishes. Zoo has 16 species of exotic bird species which help in additional attraction to the collection. The primary purpose of the zoo is successful breeding of animals in captivity, especially for exotic and endangered ones and releasing those who can survive in the wild. The Kathmandu valley has moderate type of climatic condition so native animals can adjust well in its environment. For some exotic animals, habitat manipulation is done to those who need specific climatic condition. Desert bird, Ostrich is kept in enclosure with sandy soil and proper sun warmth.

An exotic species is a species that historically lives in one places, but that has been moved to a new geographic location due to human interference. Some important exotic avian fauna present at Central Zoo of Nepal includes African Grey Parrot, African Ostrich, Emu, Golden pheasant, Guinea fowl, Java sparrow, Silver pheasant, Blue and Yellow Macaw etc. The

Ostrich was named in 1758 by Linnaeus as *Struthio camelus*, based on the Greek and Latin name (Bertram 1992). Ostrich (*Struthio camelus*) is the largest and heaviest living bird and is the only bird with just two toes and sole representatives of the order Struthioniformes (Alden *et al.*, 1996). Wild Ostriches are confined to the drier parts of Africa, generally from south of the Sahara to Cape Province, extending also to the Northern Sudan and Southern Egypt (Dingle and Shanawany, 1999). Parakeets and Parrots from Australia, Africa, Asia and the Pacific region are currently gaining in popularity as pets due to their bright plumage, small to medium size, and ease of breeding in captivity (Levine 2003). Golden Pheasants (Chinese Pheasants) are the one of the most popular species of pheasant distributed in the mountain forests of west and central China.

1.1.2 Parasitic infection of captive birds in Zoological Garden

In the natural environment, animals might have higher natural resistance against parasitic infection. Due to the change in environment and living from free condition to captive stressful condition influence the ecology and behaviors of animals thus increasing the possibility for parasitic infection (Goossensa *et al.*, 2005). Close relation among exotic and native birds in zoo plays a vital role in the transmission of parasite and parasitic disease to those species which were not normally come in close contact with these parasite and parasitic infection. Transmission of pathogens can occur in both direction from exotic ones to native or native to exotic avian species (Ajibadi *et al.*, 2010). Some parasites are host specific while other can infect a wide range of avian species. Captive birds need favorable or balanced environmental condition, free of stress and disease along with sufficient space to fly freely. Due to the lack of proper facility avian fauna may suffer from parasitic infection such as gastro-intestinal protozoan and helminth causing different clinical appearance (Lima *et al.*, 2016). When flocks of wild birds are kept in captivity in zoological gardens, the problem of parasitic infections can aggravate and pose a serious threat to endangered species, occasionally causing sudden and unexpected local declines in abundance (E-Shahawy and Elenien, 2015).

Parasitic diseases became one of the major problems causing mortality in birds and also can affect host survival rate as well as reproduction directly due to loss of blood, tissue damage and spontaneous abortion (Rao and Acharjyo, 1984). Insufficient information on disease and parasites of avian fauna is the important limiting factor for parasitic infection in many Zoo (Javaregowda, 2016). Birds can be parasitized by broad variety of endoparasites such as nematodes, trematodes, cestodes, acanthocephalans and protozoa. Parasitic worm infections may cause considerable damage to birds due to malnutrition, decreased feed conversion ratio, weight loss, lowered egg production and death in young bird. The Poor diet quality and quantity of space is likely to create boredom and frustration to the captive birds, so that they may express stereotypic behavior, which should be regarded as unfavorable and to be avoided as soon as possible. Some parasites of birds may have zoonotic potential and cause a risk to human health (Maske *et al.*, 1990) and raised public health concern (Adekunle and Olayide, 2008). Human disease can be caused by direct or indirect contact with infected animals. There

are many factors that increase the probability of zoonotic disease transmission such as time of infection and route of infection, latent period, host resistant against pathogens, population density and animal handling (Marietto *et al.*, 2008). Coccidian parasites like *Eimeria* sp. and *Isospora* sp. were commonly found in poultry, Columbiformes, Galliformes, Anseriformes occasionly on Psittaciformes and Pelicaniformes (Cole and Friend, 1999). The major gastrointestinal nematode parasites reported in avian fauna are *Ascaridia* sp., *Capillaria* sp., *Contracaecum* sp., *Heterakis* sp., *Strongyloides* sp., *Syngamus* sp., *Tetrameres* sp. Trematodes includes *Cotylurus* sp., *Echinostoma revolutum, Catatropis* sp. Cestode includes *Anomotaenia* sp., *Raillietina* sp., *Choanotaenia* sp. (Weekes, 1982).

Careful health monitoring and parasite control can reduce health risks in birds. Monitoring should be done with a veterinary surgeon and may consist of faecal sampling and examination for the presence of parasites and bacteria, including potential zoonosis such as those caused by Salmonellae and Campylobacter. During this time period birds can be treated for the presence of endoparasites and ectoparasites on advice from the attending veterinarian (Hawkins *et al.*, 2001). The performance of captive populations could be enhanced by setting the captive environment more or less as like to the natural habitat of captives (Carroll *et al*., 2014). The parasites exhibits an aggregated distribution and the removal of few heavily infected host from the host population will show the large effect on number of parasite population and decrease the impact of parasite on host population.

1.2 Objectives

1.2.1 General Objective

To identify the gastro-intestinal parasites in exotic avian fauna present at Central Zoo, Jawalakhel, Lalitpur, Nepal.

1.2.2 Specific objectives

- To determine the morphometric features of eggs, cysts and oocysts of GI parasites in exotic avian fauna for their identification.
- To determine the general prevalence of gastro-intestinal parasites in exotic avian fauna at Central Zoo.
- To determine the prevalence of helminth and protozoan parasites and also single and mixed infection of different gastro-intestinal parasites.
- To assess the management practices of exotic avian fauna at Central Zoo.

1.3 Justification of the study

Birds can transmit many pathogens such as protozoans and helminth directly or indirectly. Information about avian borne diseases and zoonotic parasites are limited and insufficient. A Few study has been carried regarding the GI parasites of avian fauna in context of Nepal which may be one of the factor in declining the population of avian fauna. The objective of this study is to provide a brief account of major parasites prevalent in avian fauna. This will help to

identify the exotic bird species wise general prevalence of GI parasites and also benefit for developing knowledge about parasitic infection in different exotic birds which will help the concerned authorities of the Central Zoo for the formulation of plans and policies regarding conservation and management. In addition, this research will also create opportunity for the further study.

2. LITERATURE REVIEW

Heavy infection by gastro-intestinal parasites were likely to be harmful for avian fauna (Parsani *et al*., 2003; Hoque *et al*., 2014). The parasitic infection can be controlled by antihelminthic therapy and management practices, but the possibility of recurrence is always there due to the stress and closer proximity of animals in Zoological Garden (Singh *et al*., 2006). Gastrointestinal diseases are an important problem of Zoo animals. Gastrointestinal parasites of Zoo animals included zoonotic species to human and raised public health concern (Maske *et al.*, 1990). Occurrence of parasites in birds kept in zoological garden might vary according to husbandry practices, diseases prophylaxis and treatment administered (lim *et al*., 2008) and parasitic infection in captive animals were caused by Ascarids, Hookworms, Trichostrongylidae, Strongylidae, *Trichuris* sp., *Capillaria* sp. and *Heterakis* sp. (Gurler *et al*., 2010). Prevention and control strategies are the best way in public health veterinary medicine of preventing infection in captive animals (Fakae and Paul, 1999).

2.1. Global context

Most health problems in Ostriches occurred during the first three months of life span with the mortality rate ranging between 30-40%. Problem of Ostrich farming and production, such as ecto and endo parasites which could negatively affect the production. *Capillaria* sp. was recorded in the faecal samples by using concentration techniques of both captive and free-living Ostriches in North east Nigeria (Ibrahim *et al.,* 2006) and also in different states of Europe (Ponce Gordo *et al*., 2002). *Codiostomum struthionis* is a parasitic nematode of the family Strongylidae measuring about 1-1.5 cm long and white in color that feeds on caecal mucus (Ederli *et al*., 2008) and these parasites were most commonly found in Ostriches (Dingle and Shanawany, 1999). Worldwide ratitie farming is a quite new field of animal production having few study on parasitic infection occurred in them. Up to the present time the most endo parasites species found in ostriches are similar in case of internal parasite species of other ratities (Karel and Daniela, 2012). Nematodes were important organism that limit the development of Ostriches. Ostriches may be infected with their own specific GI parasites as well as with ecto and endo parasites of other birds, ruminants and raccons (Eslami *et al.,* 2007) and conducted a research to identify gastrointestinal parasites in faecal samples of 254 Ostriches, collected from five farms located at different part of Iran. This study revealed presence of *libyostrongylus douglassii*, *Heterakis dispar* and Oocyst of *Eimeria* sp. In addition, Ibrahim *et al.,* (2006) conducted a research work on 100 total faecal samples of Ostriches (50 under natural condition and 50 under captive condition) at semi-arid region of north Eastern Nigeria. A single protozoan parasite *Eimeria* sp. and different helminth parasites such as *Ascaridia* sp., *Strongyloides* sp., *Strongyle* sp. and *Capillaria* sp. were identified by concentration method. The overall prevalence of gastro-intestinal parasites on captive and free living ostriches were 44 (88%) and 36 (72%) respectively. Similarly, Pesenti *et al*., (2015) observed 34 Ostriches for nematode infection in Brazil by postmortem examination. Three species of nematode were revealed as *libyostrongylus douglassii, Codoistomum struthionis* and *Libyostrongylus dentatus* with the prevalence of 82.35%, 79.41% and 5.8% respectively.

Likewise, Ederli *et al.,* (2015) collected a total of 192 faecal samples for gastrointestinal nematode examination from 13 ostriches breeding farm in the state of Rio de Janerio and examined by flotation and sedimentation method. The investigation revealed that 179 Ostriches were infected with three nematode parasites with the prevalence of 93%. The nematode species were *Libyostrongylus dentatus, Libyostrongylus douglassi* and *codiostomum struthionis.*

Trichostrongylus tanius had been detected from the caeca of Emus. This Trichostrongylid nematode were distributed in North America, Asia and Europe infecting game birds, chickens, Ducks, Turkeys, Pheasants, Ostriches and Emus (Taylor *et al.,* 2007) and also reported that the wire worm- *Libyostrongylus Douglassii* had higher prevalence of gastrointestinal parasites causing Libyostrongylosis. This parasite belongs to a genus of nematoda in the family Trichostrongylidae. Wire worm are wire like, rounded and very small measuring about 3mm long, male being 4-6mm long and female 5-6mm long. *Deletrocephalus Dimidiatus* is a strongylid nematode parasite found in small intestine of Emus. This parasite is widely distributed in Europe, North and South America. Ederli *et al*., (2014) stated that the ratitie group is composed of flightless birds such as Ostrich, Rheas, Emus, Cassowaries and Kiwis. Control of parasitic diseases were one of the serious problem in breeding of ratitie at captive farm and reported protozoan and helminth parasites could parasitized ratitie group. The reported protozoan parasites in ratitie were *Balantidium* sp., *Cryptosporidiam* sp., *Eimeria* sp., *Isospora* sp. and *Toxoplasma gondii*. Nematode parasites were *Ascaridia* sp., *Capliiaria* sp., *Codiostomum* sp., *Libyostrongylus* sp. and *Syngamus* sp. Trematode were *Fasciola* sp. and *Philophthalmus* sp. Cestode parasites infecting ratitie revealed *Davainea* sp., *Houttuynia struthionis* and *Raillietina* sp. In addition, Reissing *et al.,* (2001) conducted a research work from total 310 faecal samples of flightless bird Rhea and Emus and found overall prevalence of 42%. 97(31.3%) faecal samples were found positive for oocyst of *Eimeria* sp. Egg of *Trichostrongylus* sp. and *Capillaria* sp. were found positive in 27 (8.25%) and 6 (1.9%) respectively. Out of 130 positive faecal samples, 46 (35%) were from chicks and 84 (65%) were from adult and juveniles of Rheas.

Pheasants treated with anthelminthic increased the survival and reproductive success (Draycott *et al*., 2006) reported that in the absence of anthelminthic treatment, parasitic infection increased rapidly through March and April, whereas birds given anthelminthic treated had lower parasitic infection during the same periods. The breeding success of pheasants was significantly higher on plots provided with anthelmintic treatment. In addition, Santilli and Bagliacca, (2011) determined intestinal parasites of pheasants by using concentration method in 13 hunting areas from Italy. A total of 248 faecal samples were collected and evaluate for the presence of gastrointestinal parasites. In the faecal examination four species of Nematode parasites such as *Capillaria* sp., *Syngamus* sp., *Heterakis* sp. and *Ascaridia* sp. and single species of Protozoan parasites *Eimeria* oocyst were identified. *Ascaridia* sp. are the nematode parasite found throughout the world, which possesses a wide host range, infecting the small

intestine of Chickens, Turkey, Guinea fowl and a number of Pheasants birds, including Peafowl (Teixeira *et al*., 2012). After examination of total 12 faecal samples of Peafowl revealed 66.6% represented single or mixed nematode infection and the most frequent observed nematode eggs was Ascarid eggs. Parasites caused by helminths produced health problems in free-living and artificially raised Pheasants (Draycott *et al*., 2006). Nematode infections were the most frequently transmitted through intermediate host to the pheasant population. The investigation revealed that 41.83% and 33.03% of helminth infections had occurred in young and adult Pheasants respectively. The observed nematode species were *syngamus trachea, Ascaridia galli, Ascaridia columbae, Heterakis gallinae, capillaria Columbae and Capllaria phasianis* (Pavlovic *et al*., 2003). Similarly, Goldova *et al*., (2006) observed gastrointestinal parasites of Pheasants in confined system. A total of 1030 faecal samples were collected and examined for prevalence of gastrointestinal parasites by qualitative flotation method. 497 samples were found positive with 48.2% prevalence for intestinal parasites. five species of nematode were observed such as *Capillaria* sp. (38.4%), *Syngamus trachea* (45.8%), *Heterakis* sp. (31.7%), *Isolonche* sp. (31.8%), *Ascaridia* sp. (10.5%) and *Trichostrongylus tenius* (2.1%).

The change in environment and change in living condition from freedom to captivity influences the ecology of animals and birds that might increase the sensitivity to the disesase, especially parasitic infection. Fatal *Ascaridia galli* infection in Guinea Fowl (*Numida meleagris*) was reported from Jammu region (Sudhan *et al*., 2009). The presence of parasites in Guinea Fowl particularly in young ones resulted reduced body weight and reproductive disorder. Due to the heavy parasitic infections there may be death of Guinea fowls (Attah *et al*., 2013) and surveyed gastrointestinal helminths of 400 alimentary tract (200 from Chicken and 200 from Guinea Fowls) at Sokoto, Nigeria. The result showed that 86% of birds harbored intestinal helminth parasites. The most prevalent parasites were *Raillietina echinobothrida* (44.8%) followed by *Ascaridia numudae* (19.5%), *Heterakis gallinarum* (15%), *Ascaridia galli* (10%), *Hymenolepis carioca* (4.9%) and *Sublura brumpti* (3.2%). Similarly, Singh and Muhammed (2016) observed gastrointestinal nematodes in 15 Guinea fowls in Nigeria by postmortem examination. The high prevalence of 80% nematode infection was observed among sampled examination. The observed nematodes were *Ascaridia galli* (67%), *Heterakis gallinarum* (40%), and *Strongyloides ovium* (20%). In addition, Singh *et al*. (2006) collected a total of 252 (102 from Turkeys and 150 from Guinea fowls) faecal samples to analyze the presence of coccidian infection at Izatnagar were examined by Flotation technique. Result revealed that 5% of Turkey and 46% of Guinea fowls were infected with *Eimeria meleagrimitis* and *Eimeria gorakhpuri* respectively.

Prathipa *et al*., (2013) investigated the prevalence of gastrointestinal parasites of captive Psittacine birds at Chennai. A total of 250 faecal samples were collected and examined by centrifugal sedimentation and floatation technique. The results revealed the presence of endoparasites such as *Ascaridia* sp. (11.20%), *Capillaria* sp. (20%), *Strongyloides* sp (2%), *Strongyle* sp. (1.60%) and oocyst of *Eimeria* sp. (10.40%). The prevalence of mixed infections

were found to be (11.60%). Similarly, Ramisz *et al.* (2007) conducted a study to determine parasitic specific composition, prevalent and intensity of infection. A total of 95 faecal samples were collected from 5 different species of Parrot such as Budgeriger (n=36), Cockatiels (n=21), Grey Parrot (n=18), Eastern Rosella (n=10) and Senegal Parrot (n=10) were examined by Willis-Schlaff and McMaster method. Results showed that two protozoans (Isosporidae and Eimeridae) and 3 nematodes (Ascarididae, Capillaridae and Heterakisdae) were obtained. All the examined Parrots were positive for coccidian Oocyst. Cockatiels and Senegal Parrot were free from nematodes and other three species of parrot were infected with one or more nematode parasites. *Ascaridia platycerci* was present on Budgeriger, Gery Parrot and Eastern Rosella. *Heterakis gallinarum* was recorded only in Grey parrot. Eastern Rosella found to have highest intensity of infection with nematode of Ascaridae and Capillaridae with egg per gram 1242 and 2480 respectively.

Parasitic disease were the major health problem affecting Passerine birds that caused Subclinical alteration or even death of the birds subjected to stress. A research was conducted to detect helminth eggs and protozoan Oocyst in the faecal samples from birds of the order Passeriformes in Brazil. The prevalence of protozoan oocysts 93.68% were observed higher than helminth parasites 10.34%. The observed helminth parasites were *Trichostrongylus* sp., *Ascaridia* sp., *Trichuris* sp. and protozoan include oocysts of *Isospora* sp. (Matos *et al*., 2016). In the study of helminth fauna of Passerines in Netherlands (Borgsteede *et al*., 2000) collected faecal samples of total 210 birds and revealed 138 (67.7%) being infected with helminths. Of these birds, 22.4% were infected with Trematodes, 39% with Cestodes, 42.5% with Nematodes and 17% with Acanthocephalans. Altogether 29 species of helminths were identified, among them 11 species of Trematodes, 5 Cestodes, 11 Nematodes and two Acanthocephalans.

Gastrointestinal parasites of avian fauna at different Zoological Garden

Zoological Garden is the home to numerous animals like mammals, birds, reptiles and fishes. Close relation among exotic and native birds in zoo play a vital role in the transmission of parasite and parasitic disease to those species which were not normally come in close contact with these parasite and parasitic infection (Ajibadi *et al.*, 2010). Varadharajan. A and Kandasamy. A (2000) conducted a survey of GI parasites of wild animals in a Mini Zoo and reported 58% were found positive for helminth parasitic infection and 6% positive for intestinal protozoan parasites. Among helminthic infections, the prevalence of nematode infections was higher as compared to cestode and trematodes. *Strongyle* sp., *Trichuris* sp., *Strongyloid* sp. as well as Coccidian oocysts were present in case of herbivores and *Toxoplasma* sp. and *Ancylostoma* sp. were found in case of carnivores. Among the infected animals, herbivores showed multiple infections with more than one helminth parasites. Similar type of research was conducted at Nanda Van Zoo to observe the prevalence of gastro-intestinal parasitic infection in 210 faecal samples of captive wild animal found positive for 97 faecal samples for different helminth parasites. Among helminthes, the prevalence of nematode and Cestode were

97.94% and 8.24% respectively. The prevalence of GI parasites were found higher in primates (60%) followed by herbivores (45%) and carnivores (45.2%) during the study by microscopical examination of faecal samples (Thawait *et al.,* 2014). In addition, Borghare *et al.* (2009) examined a total of 30 faecal samples of captive wild pigeons at Maharajbagh Zoo, Nagpur by concentration method. Three species of helminths parasites was isolated such as *Capillaria* sp., *Ascaridia* sp. and *Heterakis* sp. with prevalence of 56.66%, 76.66% and 16.66% respectively during the study period. In 17 samples mixed parasite infection were recorded with either *Ascaridia* sp. and *Capillaria* sp. or with *Ascaridia* sp. and *Heterakis* sp.
In addition, Parsani *et al*., (2003) performed research by concentration method to determine the prevalence of parasites in different captive birds from Kamla Nehru Zoological garden, Kankaria Zoo. A total of 138 faecal sample were examined and revealed 62 (44.93%) sample were positive for one or more parasites. They found three species of parasites including 2 species of nematode and one species of protozoan and absence of cestodes and trematodes. The parasites were *Ascaridia* sp. *Capillaria* sp. and *Eimeria* sp. observed in 25 (71.42%), 13 (37.14%) and 53 (85.48%) samples respectively.

The high prevalence of gastrointestinal parasites without clinical signs of disease or mortality could be considered as subclinical infections (Mir *et al*., 2016) and examined a total of 31 faecal sample found overall prevalence 20 (68%) from 8 species of captive animals in Bir Moti Bagh Mini Zoo, Punjab. These samples were examined by concentration method. *Strongyles* were the most prevalent parasite observed (67%) followed by Coccidia (38%), *Trichuris* sp. (19%), *Strongyloides* sp. (14%), *Ascaridia* sp. (10%) and *Capillaria* sp. (10%) show equal prevalence. Cestode and Trematodes were absent during the study. Likewise, Patel *et al.,* (2000) reported 48% positive for parasitic infection from total of 106 faecal sample of captive birds through the process of sedimentation and flotation technique. *Ascaridia* sp. and *Capillaria* sp. were helminth parasite found with 20.75% and 13.20% respectively and single protozoan parasite was *Eimeria* sp. with 17.92% prevalence. The egg size of *Ascaridia* sp. ranged from 64-78x32-45 micrometer, for *Capillaria* sp. egg size were 44-60x22-24 micrometer and oocyst of *Eimeria* sp. were ranged from 15-20x10-16 micrometer. Similarly, Momin *et al.* (2001) observed the parasitic infections in 28 captive birds at Sakkarbagh Zoo. Faecal sample were collected to determine the gastrointestinal parasites through qualitative and quantitative faecal examination. The overall prevalence of gastrointestinal parasites was revealed 20 (71.43%). Egg of nematodes such as *Ascaridia* sp. (7.69%), *Capillaria* sp. (38.46%), *Trichostrongylus* sp. (7.69%) and *Hererakis* sp. (7.69%). Protozoan parasites such as oocyst of Coccidia (*Eimeria* sp.) was observed in (50%).

The performance of captive populations could be enhanced by setting the captive environment more or less as like to the natural habitat of captives (Carroll *et al*., 2014). Otegbade and Morenikeji, (2012) performed the research study to identify the prevalence of GI parasites and parasitic infection of avian fauna in five Zoological Garden in South –West Nigeria. The

overall prevalence of GI parasite was 21.9%. The highest prevalence (100%) of infection was recorded in Unilag Zoo followed by Unaab Zoo (75%) and Ilorin Zoo (24%). A total of five parasites species were recorded among them two were Protozoans (Coccidian and *Balantidium* sp.) and three were Nematodes (*Capillaria* sp., *Ascaridia* Sp. and *Strongyloides* sp.). Helminthes parasites were more prevalent than Protozoans. Helminthes parasites comprised mainly of Nematode. *Capillaria* sp. was the most prevalent followed by *Ascaridia* sp. and coccidian. Heavy infection with helminths can also cause obstruction of the intestine with the mass of worms. Lima *et al.,* (2016) detected the gastrointestinal parasites of exotic birds loving in captivity in the state of Sergipe, North Eastern Brazil. A total of 362 faecal ample were analyzed by direct examination and Willis-Mollay technique. Result showed that egg of *Heterakis* sp., *Amodostomum* sp., *Trichostrongylus* sp., *Ascaridia* sp., *Capillaria* sp. and unsporulated oocysts were detected. Likewise, Papini *et al.,* (2012) use direct microscopical examination and concentration flotation technique. Faecal sample were individually collected from Pet (n=63) and Zoo (n=83) birds in Italy**.** Overall prevalence of endoparasites infection in zoo birds and pet birds were (42.2%) and (27%) respectively. The identified GI parasites were Capillarids (8.9%) with higher prevalence followed by *Ascaridia* (6.8%), *Strongyle* (5.5%), *Coccidia* (4.1%), *Cryptosporidium* (4%) and *Porrocaecum* (2.7%). In addition, Cordon *et al.* (2009) conducted research in birds of Almunecar ornithological garden. A total of 984 faecal sample and 41 blood sample were collected from psittacidae, Phasianidae, Anatidae and Cacatuidae. (51.6%) of faecal sample were found positive for one or more intestinal parasites and 26.8% of blood sample were found positive for blood parasites. The intestinal protozoan parasites detected were Coccidian such as *Cyclospora* Sp. (4.5%), *Eimeria* Sp. (4.1%), and *Isospora* sp. (2%). Helminth comprise of only nematodes such as *Capillaria* sp. (10.1%), *Ascaridia* sp. (4.9%) and *Heterakis* sp. (4.9%). Blood protozoan parasite detected were *Haemoproteus* sp. (17%) and *Plasmodium* sp. (7.3%). Sahoo et al., (2009) assessed the prevalence of GI parasites of captive birds of Nandankanan Zoo. A total of 44 species of birds were screened for prevalence of GI parasitic infections during three seasons. The overall prevalence rate was 29.54%. The nematode parasites were diagnosed in 23.10%, 18.97%, 6.90% and 3.54% of birds respectively. In addition, Tigin *et al*., (1989) during investigation of the helminthological status of some birds in the Ankara Zoo. The birds had been found infected with *Heterakis* sp., *Strongyloides* sp., *Ascaridia* sp, *Capillaria* sp. and *Trichostrongylus* sp.

Because of space limitation, those animals kept in captive often infected to parasitic infection which cause a serious threat to endangered and exotic species. Gurler *et al*., (2010) described the occurrence of helminth infections in Zoo animals that were mainly caused by *Ascaris* sp., *Trichostrongylus* sp., *Strongyle* sp, *Heterakis* sp. and *Strongyloides* sp. Hoque *et al*., (2014) conduct a survey of gastrointestinal parasitic among domestic and wild birds in Bangladesh by faecal examination. A total of 304 faecal samples were collected from indigenous domestic ducks, 40 faecal sample from resident wild birds and 35 from migratory birds. 25%, 20% and 40% of faecal sample were found positive for indigenous domestic ducks, residential wild birds and migratory birds respectively. Prevalence of parasitic infection was significantly higher in

indigenous domestic ducks collected during summer (39%) than winter (22%). Helminth parasite eggs detected in faecal of both in domestic and wild birds were *Ascaridia* sp., *Capillaria* sp. and *Heterakis* sp. In addition, Edosomwan and Ogbonnia, (2014) examined 52 wild birds revealed overall prevalence of 23.07% by gastro-intestinal tract examination. The examined bird species were *Turdus pelios, Passer griseus, Chinnyris coccingatrus, Ploceus cuculatus* and *Apus affinis.* The helminth parasites revealed include five species of nematode such as *Ascaridia gali, Heterakis gallinarum, Subulura brumpti, Capillaria caudinflate* and *Dispharynx nasuta* and two species of cestode such as *Choanotaenia infundibulum* and *Raillietina tetrogona*. Nematodes were most abundant and showed total prevalence of (19.23%) followed by cestode (3.85%).

2.2 In context of Nepal

Parasitic infection is the major cause of illness but in context of Nepal scanty study has been carried out regarding to gastrointestinal parasites of exotic avian fauna present at zoological garden. Poudel, (2013) conducted a study with 92 faecal samples in Ostriches of order Struthioniformes farmed at Gangolia VDC-1, Rupandehi and examined by sedimentation and Flotation technique. In Nepal, 50-55 percent humidity and 90° to 99° F temperature is required for Ostrich farming. Ostriches are susceptible to various diseases like coccidiasis, nematodiasis, respiratory diseases, bacterial, fungal and viral infections. The result revealed overall 86.96% prevalence of gastrointestinal parasites. Five protozoan species and one species of unidentified cestode and four species of nematodes were recorded. Nematode parasites were highly prevalent followed by protozoan parasites and cestodes. The determined protozoan parasites were *Entamoeba* sp., *Eimeria* sp., *Isospora* sp., *Balantidium* sp. and *Histomonas* sp. Nematode parasites were *Ascaridia* sp., *Libyostrongylus* sp., *Sarratospiculum* sp. and *Codiostomum* sp. Likewise, Infection with *Ascaridia galli* in White Rumped Vulture and Slender Billed Vultures at Chitwan district was also reported (Gupta and Pandey, 2007). In addition, Gurung, (2016) examined helminth parasites observed by faecal examination of *Columba* sp. of the order columbiformes at Pokhara. The recorded Helminth parasites were *Capillaria* sp. (31.67%), *Ascaridia* sp. (21.66%), *Echinostoma* sp. (7.50%), *Syngamus* sp. (5.83%), *Hymenolepis* sp. (3.33%), *Heterakis* sp. (2.50%) and (19.16%) of coccidians. A study was done with 480 dropping samples of *Gallus gallus* of order Galliformes showed a positive sample of 125 for coccidiosis that accounted for 25% of prevalence. Among the positive cases the highest prevalence of coccidiosis occurred in the month of March i.e. 50% and no any positive samples were detected in the month of November and December. Summer and spring season accounted the highest prevalence of coccidiasis (33%) followed by winter season (23%) and the least (14%) in the autumn season (Adhikari *et al*., 2008).

3. MATERIALS AND METHODS

3.1 Study area

The Central Zoo of Nepal is situated at Jawalakhel, Lalitpur within the geographical coordinates of 27°40′23″ North latitude and 85°18′39″ East longitude. It was established in 1932 A.D. by Rana Prime Minister Juddha Shamsher JBR as private zoo. It is expanded with an area of six hectares. Due to some political issue Central Zoo came under ownership of government of Nepal in 1951 A.D. National Trust for Nature Conservation (NTNC) aims to develop the Central Zoo as a center of excellence for wildlife research and conservation education. The study involves 16 species of exotic birds present at Central Zoo (Table 1).

Table 1: Classification of surveyed exotic birds present at Central Zoo.

Common Name	Scientific Name	Order	Family
African Ostrich	*Struthio camelus* Linnaeus, 1758	Struthioniformes	Struthionidae
Emu	*Dromiaus novaehollandiae* (Latham, 1790)		Dromiidae
African Grey Parrot	*Psittacus erithacus* Linnaeus,1758	Psittaciformes	Psittacidae
Budgeriger	*Melopsittacus undulates* (Shaw, 1805)		
Cockatiel	*Nymphicus hollandicus* (Keer, 1792)		
Peach Face Love Bird	*Agapornis roseicollis* (Vieillot, 1818)		
Salmon-crested Cockatoo	*Cacatua moluccensis* (Gmelin, 1788)		
Sulphur-crested Cockatoo	*Cacatua galerita* (Latham, 1790)		
White Cockatoo	*Cacatua goffiniana* (Muller, 1766)		
Blue and Yellow Macaw	*Ara ararauna* (Linnaeus, 1758)		
Guinea Fowl	*Numida meleagris* (Linnaeus, 1758)	Galliformes	Numididae
Golden Pheasant	*Chrysolophus pictus* (Linnaeus, 1758)		Phasianidae
Lady Amherst Pheasant	*Chrysolophus amherstiae* (Leadbeater, 1829)		
Reeves's Pheasant	*Syrmaticus reevesii* (Gray, 1829)		

Silver Pheasant	*Lophura nycthemera* (Linnaeus, 1758)		
Java Sparrow	*Lonchura oryziora* (Linnaeus, 1758)	Passeriformes	Estrildidae

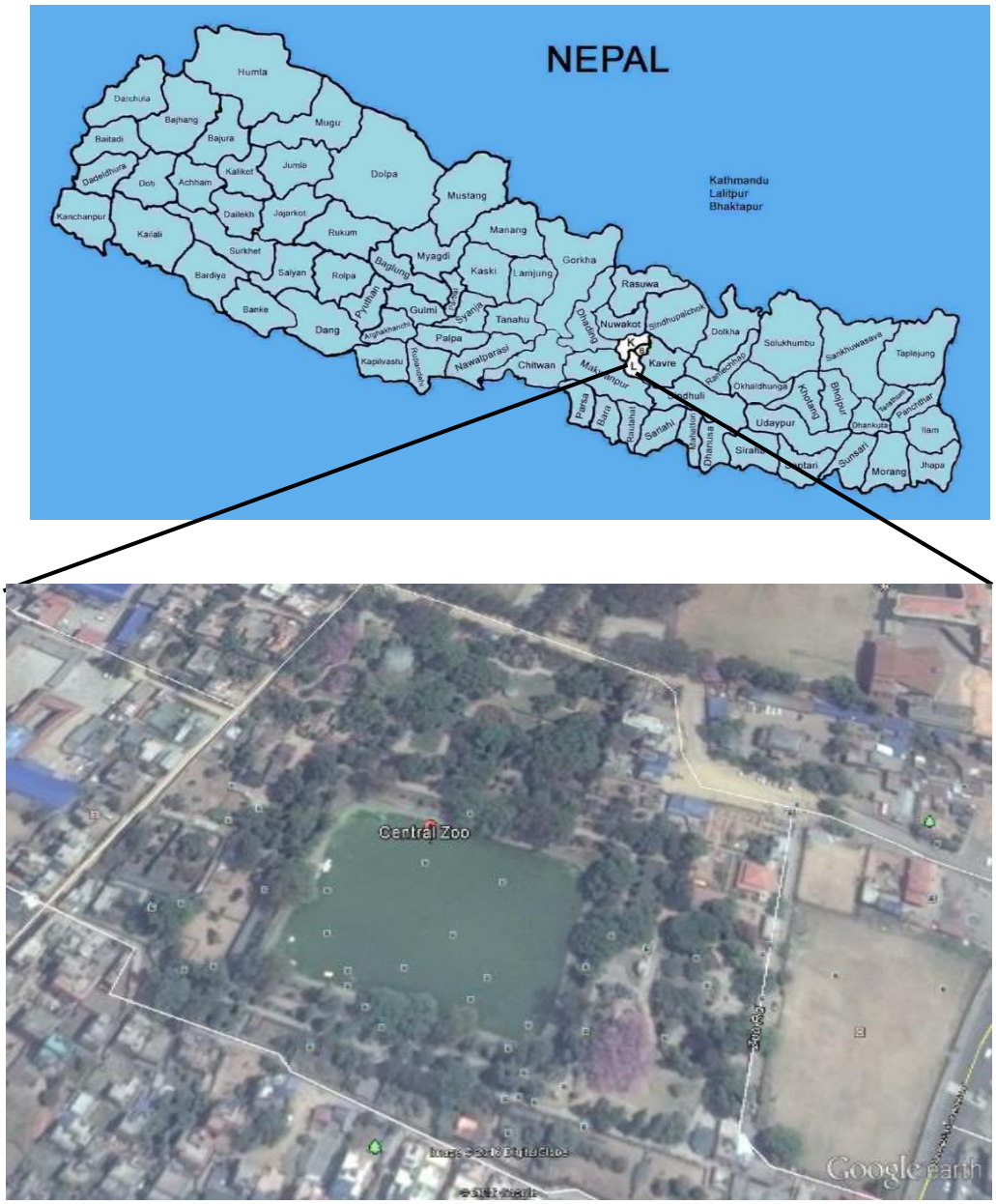

Figure 1: Map of study area; Central Zoo, Jawalakhel, Lalitpur, Nepal. (Source: maps.google.com)

3.2 Materials

3.2.1 Materials for field

Apron, Plastic Sterile Containers, Gloves, Medicate handplast and Spatula

3.2.2 Materials for laboratory

Electric microscope, Stage micrometer, Ocular micrometer, Centrifuge tubes, centrifuge machine, Gloves, Slides, Coverslips, Beakers, Volumetric Flask, Apron, Cotton, Tea strainer, Glass rod, Watch Glass, Rack and Toothpick

3.2.3 Chemical reagents

2.5% Potassium dichromate, Iodine solution, NaCl solution and Methylene Blue

3.3 Research design

This research study was designed to identify the gastrointestinal parasitic infection in exotic avian fauna in Central Zoo, Jawalakhel, Lalitpur, Nepal. It comprises:-

a) Identification of exotic avian fauna of Central Zoo by direct observation.
b) Collection of 3-5 gram faecal sample in sterile plastic container at morning time which was preserved in 2.5% Potassium Dichromate solution.
c) Examination of faecal samples by using concentration techniques (flotation and sedimentation techniques).
d) Identification and measurement of egg, cyst and oocyst of parasite by using ocular and stage micrometer.
e) Interview was performed for key informants of bird section at Central Zoo to assess the management practices.

3.3.1 Study period

The study was carried out from April 2017 to December 2017.

3.3.2 Sample size

A total of 100 faecal samples from 16 exotic bird species were collected from Central Zoo, Jawalakhel, Lalitpur, Nepal during the third week of April 2017 AD. The total individual population of 16 exotic bird species were 190. The collected samples is 52.63% from total population.

3.3.3 Samples collection

For this research work, altogether 100 faecal samples were collected from 16 species of exotic avian fauna present at Central Zoo. The samples were collected with the assistance of zookeeper of bird section before routine cleaning of cages. About 3-5 gm of faecal samples werw collected in clean, sterile vial with the help of a spatula wearing gloves and mask then filled with 2.5% potassium dichromate solution. All the samples collected were labeled properly. The same collection process was repeated for all collected faecal samples. After that, vial was kept in air tight cool box.

3.3.4 Preservation of faecal samples

Collected faecal samples of exotic bird species were preserved in 2.5% potassium dichromate solution (2.5gm of potassium dichromate powder dissolved in one liter of distilled water). Potassium dichromate help in maintaining morphology of protozoan Oocysts and cysts and prevent further development of helminth eggs and larva.

3.4 Laboratory Examination

In the lab of Central Department of Zoology preserved faecal samples were proceeded for microscopic examination of ovum, oocysts and cyst which were examined by iodine wet mount method and concentration procedures which included floatation and sedimentation methods.

3.4.1 Iodine wet mount

About 1-2 gm 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 electrical microscope at 10X and 40X (Soulsby, 1965). This technique is generally used for the detection of cysts of protozoan parasites.

3.4.2 Flotation method (Dryden *et al*., 2005)

Cestode and Nematode eggs present in bird faecal were detected through flotation technique. This technique ensures the eggs float in the floatation liquid, which helps to identify the eggs. About three gram of faecal sample was put in a clean beaker and 20 ml of distilled water was added then with the help of mortar it was mixed carefully. About 15 ml solution was put into centrifuge tube which was centrifuged at 2,000 rpm for five minutes. Tube's water was changed by saturated sodium chloride solution and again centrifuged to develop convex surface at the top of tube. To stain one drop of methylene blue was added for few minutes at the top where cover slip could be placed. After that, coverslip was removed and kept on slide which was observed under electrical microscope at 10X and 40X. Photos of eggs and cysts was taken and identified on the basis of egg's color, shape and size.

3.4.3 Sedimentation method (Dryden *et al*., 2005)

Trematode eggs present in bird feces were detected through sedimentation technique. It provides a better result as the eggs of trematode are bit heavier than cestode and nematode, sediments of centrifuged contents was taken for eggs detection. Saturated Nacl solution was removed gently after examination of floated portion from the centrifuge tube. Sediment content was poured in clean watch glass and that was stirred gently for mixing. From the mixture one drop was taken to prepare a slide. The faecal sample was stained with iodine wet mount's solution which was then examined under electric microscope at 10Xx10X and 10Xx40X objectives to detect eggs of protozoan, helminths, protozoan's trophozoites or cysts of gastrointestinal parasites.

3.4.4 Interview of key informants

A semi structured questionnaire was used as method for collecting data. Interview was conducted with key informants of bird section at Central Zoo to assess the management practices for captive birds.

3.5 Measurement of eggs, oocysts and cysts

Eggs, oocysts and cyst of parasites were measured by using ocular and stage micrometer. The calibration factor was found to be 10.35µm for 10X and 2.23µm for 40X.

3.6 Identification of eggs, oocysts and cysts

Eggs, oocyst and cysts were identified by using books of Soulsby (1982), Zajac and Conboy (2012) and also from internet sources on the basis of morphological characters.

3.7 Data analysis and interpretation

The data was recorded on the basis of laboratory examination. Firstly, the recorded data were coded and entered in MS Excel 2013. Data were statistically analyzed using Chi-square test performed by "R" version 3.3.1 software packages. Percentage was used to calculate prevalence. In all cases 95% confidence interval (CI) and $p<0.05$ was considered for statistically significant difference.

4. RESULTS

4.1 General prevalence of GI parasites in exotic avian fauna of Central Zoo

Out of 100 faecal samples examined, 54 faecal samples were positive for one or more species of GI parasites, showing 54% prevalence of parasitic infection and 46% samples were found negative for GI parasites.

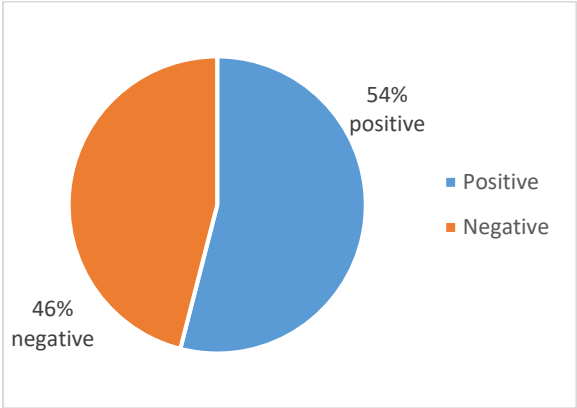

Figure: 2 Overall prevalence of GI parasites.

4.2 General prevalence of specific GI parasites

Out of 100 total samples examined, exotic birds of Central Zoo were infected with one protozoan parasite and four helminth parasites which were microscopically examined by using direct smear method and concentration technique (sedimentation and floatation). *Eimeria* sp. was the single recorded protozoan parasite. Among helminth parasites, *Ascaridia* sp. showed the highest prevalence followed by *Trichostrongylus* sp., *Strongyloides* sp. and *Codiostomum* sp. All these helminthes parasites belongs to phylum Nematoda. Cestodes and Trematodes were not found during the study.

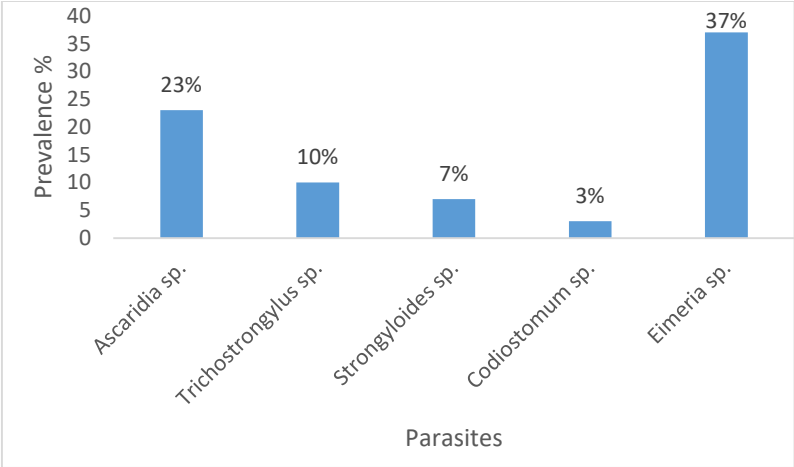

Figure 3: Prevalence of specific GI parasites.

4.3 Morphometric features of eggs, cysts and oocysts of GI parasites of exotic avian fauna at Central Zoo

Table: 2 Morphometric features of eggs, cysts and oocysts of specific GI parasites

S.N	Photo no.	Parasites	Size (µm) of cyst and eggs (10X×40X)	Size (µm) by Soulsby, 1982	Morphological characters
Protozoan					
1.	8	*Eimeria* sp.	(20.7)x (18)	(10-40)x(10-30)	Eggs were elongated oval shaped, having prominent cyst wall with or without micropyle and contain morulla which is located centrally or sub-centrally filled up.
Helminthes					
2.	9	*Ascaridia* sp.	(78)x (54)	(68-90)x((40-60)	Eggs were nearly spherical, yellowish brown, granular content and unsegmented, thick aleveolated albumious shell.
3.	10	*Trichostrongylus* sp.	(115)x (62.4)	(70-108)x(30-40)	Eggs were oval or kidney bean shaped with thin and transparent outer shell and wrinkled inner membrane, shell and embryonic mass is relatively conspicuous. One side is more rounded than other or somewhat both side rounded.
4.	11	*Strongyloides* sp.	(71)x (66)	(80-95)x(45-55)	Eggs were oval with rounded edges or ellipsoidal, thin shell and contain fully developed larvae that can be seen under low power.
5.	12	*Codiostomum* sp.	(38)x (29)	(20-40)x(20-30)	Eggs were rounded and thin shelled.

4.4 Exotic bird species wise general prevalence of GI parasites

African Ostriches, African Grey Parrot, Guinea fowl, Reeves's Pheasant and Salmon Crested Cockatoo revealed the highest prevalence (100%) of gastrointestinal parasitic infection followed by Emus (75%), Blue and Yellow Macaws (66.66%), Cocatiels (60%), Golden Pheasants (58.82%). Budgerigers show higher prevalence of gastrointestinal parasites followed by Silver Pheasants. Lady Amherst Pheasants and Peach faced Lovebirds show equal

prevalence (40%). Java sparrow, Sulpher-Crested Cockatoo and White Crested Cockatoos also show equal prevalence 33.33%.

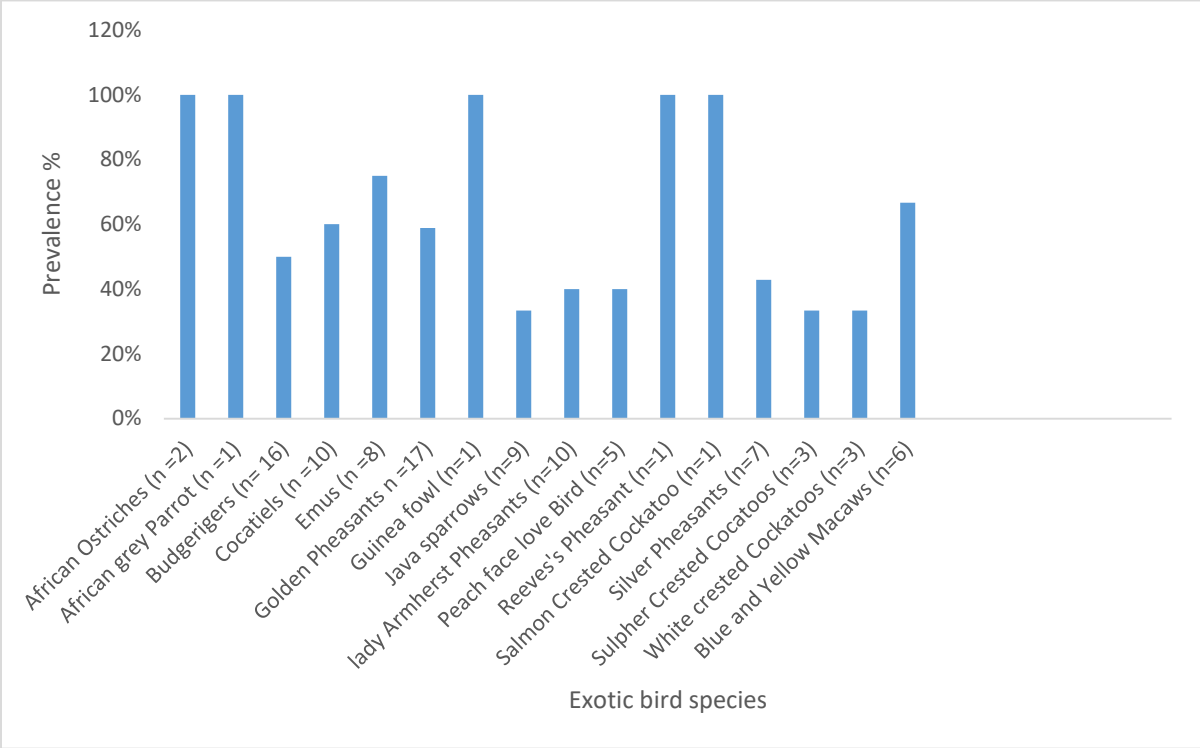

Figure 4: Exotic bird species wise general prevalence of GI parasites.

Table: 3 Parasite occurrence in the order Psittaciformes

The total of eight species of exotic birds belonging to Psittaciformes order are present in the Central Zoo. Altogether 45 faecal samples were examined from Psittaciformes, 24 faecal samples were found positive for different GI parasites. *Ascaridia* sp. recorded on Blue and Yellow Macaws revealed the highest prevalence followed by White Cockatoos, Cockatiels and Budgerigars. *Trichostrongylus* sp. were found positive in Cockatiels and Blue and Yellow Macaws. *Strongyloides* sp. were found positive in Cockatiels and Blue and Yellow Macaws. Coccidian parasite (*Eimeria* sp.) were found positive in all birds except White Cockatoos.

Scientific Name	Common name	No. of samples examined	No. of positive samples	Number of positive samples and percentage occurrence				
				A	T	S	C	E
Psittacus erithacus	African Grey parrot	1	1	_	_	_	_	1 (100%)
Melopsittacus undulates	Budgerigars	16	8	1 (6.25%)	_	_	_	7 (43%)
Nymphicus hollandicus	Cockatiels	10	6	2 (20%)	2 (20%)	1 (10%)	_	3 (30%)

Agapornis roseicollis	Peach face love Bird	5	2	_	_	_	_	2 (40%)
Cacatua moluccensis	Salmon Crested cockatoo	1	1	_	_	_	_	1 (100%)
Cacatua galerita	Sulphur-Crested Cockatoos	3	1	_	_	_	_	1 (33.33%)
Cacatua goffiniana	White Cockatoos	3	1	1 (33.33%)	_	_	_	_
Ara ararauna	Blue and Yellow Macaws	6	4	3 (50%)	1 (16%)	1 (16%)	_	2 (33.33%)

A= *Ascaridia* sp., T= *Trichostrongylus* sp., S= *Strongyloides* sp., C=*Codiostomum* sp. and E=*Eimeria* sp.

Table: 4 Parasites occurrence in the order Struthoiniformes

The total of two species of exotic birds belonging to Struthioniformes order are present in the Central Zoo. Altogether 10 faecal samples were examined from Struthioniformes, eight samples were found positive for different GI parasites. *Strongyloides* sp. was found positive only in Emus but other Coccidian parasite (*Eimeria* sp.) and helminths such as *Ascaridia* sp., *Trichostrongylus* sp., and *Codiostomum* sp. were revealed positive for both Ostriches and Emus.

Scientific name	Common name	No. of samples examined	No. of positive samples	Number of positive samples and percentage occurrence				
				A	T	S	C	E
Struthio camelus	African Ostriches	2	2	1 (50%)	1 (50%)	_	1 (50%)	1 (50%)
Dromiaus novaehollandiae	Emus	8	6	5 (62.5%)	2 (25%)	2 (25%)	2 (25%)	5 (62.5%)

A= *Ascaridia* sp., T= *Trichostrongylus* sp., S= *Strongyloides* sp., C=*Codiostomum* sp., and E=*Eimeria* sp.

Table: 5 Parasites occurrence in the order Galliformes

The total of five species of exotic birds belonging to Galliformes order are present in the Central Zoo. A total of 36 faecal sample were examined from Galliformes, 19 samples were found positive for different GI parasites. *Ascaridia* sp. revealed most prevalent parasite infecting all Galliformes. *Trichostrongylus* sp. were found positive in both Golden and Silver Pheasant. *Strongyloides* sp. were found positive in Golden and Lady Amherst Pheasants.

Scientific name	Common name	No. of samples Examined	No. of positive samples	Number of positive samples and percentage occurrence				
				A	T	S	C	E
Numida meleagris	Guinea fowl	1	1	1 (100%)	_	_	_	_
Chrysolophus pictus	Golden Pheasants	17	10	4 (23.5%)	3 (17.64%)	2 (11.76%)	_	7 (41%)
Chrysolophus amherstiae	Lady Amherst Pheasants	10	4	1 (10%)	_	1 (10%)	_	2 (20%)
Syrmaticus reevesii	Reeves's pheasant	1	1	1 (100%)	_	_	_	_
Lophura nycthemera	Silver Pheasants	7	3	2 (28.5%)	1 (14.28%)	_	_	2 (28.57%)

A= *Ascaridia* sp., T= *Trichostrongylus* sp., S= *Strongyloides* sp., C=*Codiostomum* sp., and E=*Eimeria* sp.

Table: 6 Parasites occurrence in the order Passeriformes

Single species of exotic birds belonging to Passeriformes order is present in the Central Zoo. Altogether nine faecal sample were examined from Passeriformes, three samples were revealed positive for different GI parasites. Protozoan parasite (*Eimeria* sp.) and single helminth (*Ascaridia* sp.) was found positive for Java sparrow.

Scientific name	Common name	No. of samples examined	No. of positive samples	Number of positive samples and percentage occurrence				
				A	T	S	C	E
Lonchura oryziora	Java sparrow	9	3	1 (11.11%)	_	_	_	3 (33.33%)

A= *Ascaridia* sp., T= *Trichostrongylus* sp., S= *Strongyloides* sp., C=*Codiostomum* sp., E=*Eimeria* sp.

4.5 Prevalence of helminth and protozoa parasites

Among the total examined samples, 33 were found positive with helminth parasites, whereas 37 samples were seen positive with protozoan parasites. Statistically, the difference in prevalence of helminthes and protozoan parasites was found to be insignificant (p=0.656, χ^2 = 0.197).

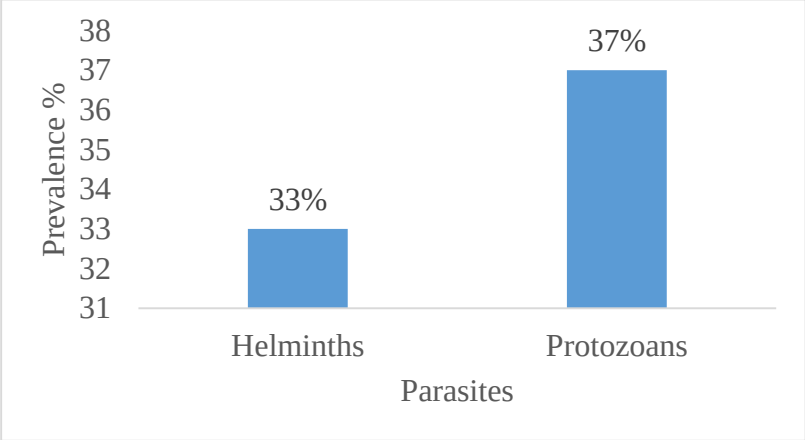

Figure: 5 Prevalence of different helminthes and protozoan parasites

4.6 Prevalence of single and mixed infection

In the present study, single and mixed type of parasitic infection were encountered in exotic avian fauna present at Central Zoo. Out of 100 total samples, single infection was found to be higher than mixed infection. Statistically, the difference in prevalence of single and mixed infection was found to be significant (P= 0.034, χ^2 = 4.481).

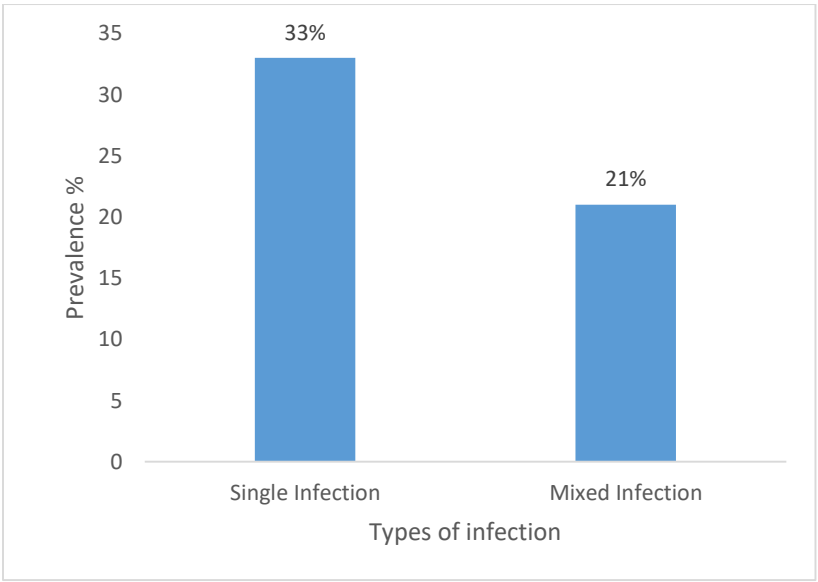

Figure 6: Prevalence of single and mixed infections

4.7 Types of mixed infection

Out of 100 total faecal samples examined, 21 samples were found positive for mixed infection. Among mixed infections, double infection and multiple infection were found in 16 and five faecal sample respectively. Double infection showed the higher prevalence rate than the multiple infection in this study. Statistically, the different in prevalence of double and multiple infection was found to be significant difference (P= 0.002028, χ^2 = 9.523).

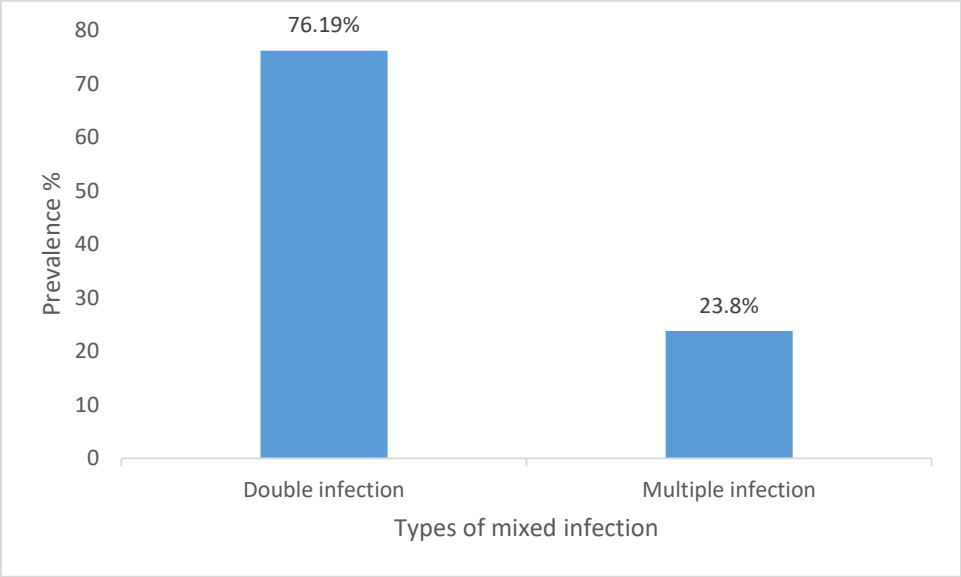

Figure 7: Prevalence of double and multiple infections

4.8 Management practices in the study area based on key informants

A questionnaire survey was conducted with key informants of bird section at Central Zoo to assess the management practices. The information obtained from them were almost similar although the questions were asked separately.

- All key informants of bird section were familiar with exotic birds and can differentiate exotic and endemic birds present in the Zoo.
- The initial action taken soon after the exotic birds bringing inside the zoo was keeping them in separate cages for one to two months for well adaptation.
- The food that were supplied to birds were brought through tender process from Kalimati vegetable market and cleaned well in kitchen room before chopped it into small pieces. Food and water were supplied by assistant zookeepers in morning time at 10:30 to 11:30 am. Visitors are not allowed to feed birds.
- Tap water was the source of water given to the birds. Birds fed according to their food preference and dietary requirements which are categorized in three types as flesh and eggs, fruits and vegetables and cereals.
- Feeders and utensils were not regularly cleaned and the drainage system were not properly managed that increase the occurrence of parasite and parasitic disease. Chemical disinfectants such as Dettol, Porcelains were used during cleaning.

- Many foraging birds such as sparrow, pigeon and crows enter inside the cage and play an important role in transmission of parasites.
- For the maintenance of proper health of animals, animal hospital has been established. The health of birds were regularly monitored by assistant zoo keeper during feeding time.
- The birds were suffered by protozoan disease characterized by diarrhea more frequently. In emergency case physical examination, faecal examination, blood examination, vaccination were done in the Central Zoo to prevent captive birds from various disease.
- The hygiene of kitchen is good. Daily use of mask, gloves, boots and apron were the safety measure adopted by zookeepers during entering into the cage. Sometime, the birds were seen dead in the cage by parasitic disease. Such dead birds were postmortem on the veterinary lab of Central Zoo by veterinary doctor then buried in the soil far away from cage.
- The area of zoo is limited and hence to keep the population of animals with in the carrying capacity of zoo, breeding control is done either by sterilization or separating male and females during breeding season.
- Anthelminthic drugs were provided twice a year regularly only for captive birds.
- Deworming programme is found to be effective because the birds kept inside the cage are active and death rate is very low but the possibility of reappearance of parasitic infection is always there due to stress and also due to closer proximity with other foraging birds that directly transmit parasites.

Photos of study area and laboratory examination

Photo 1: with zookeeper of bird section.

Photo 2: Cutting and separating foods in kitchen room

Photo3: Transferring food to feed captive birds

Photo 4: Foraging birds inside the cage of Emus

Photo 5: Foraging birds feeding foods of captive birds

Photo 6: Preservation of faecal samples at CDZ lab

Photo 7: Running the centrifuge machine

Photo 8: Microscopic examination

Oocyst and eggs of GI parasites of exotic birds at Central Zoo under 10Xx40X magnification

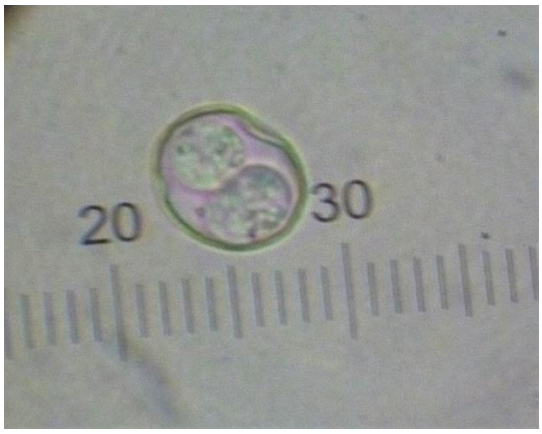

Photo 9: Oocyst of *Eimeria* sp. (20.7x18 µm)

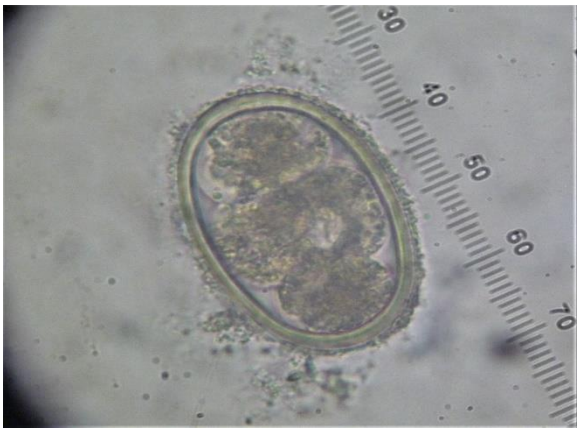

Photo 10: *Ascaridia* sp. egg (78x54 µm)

Photo 11: *Trichostrongylus* sp. egg (115-62.4 µm)

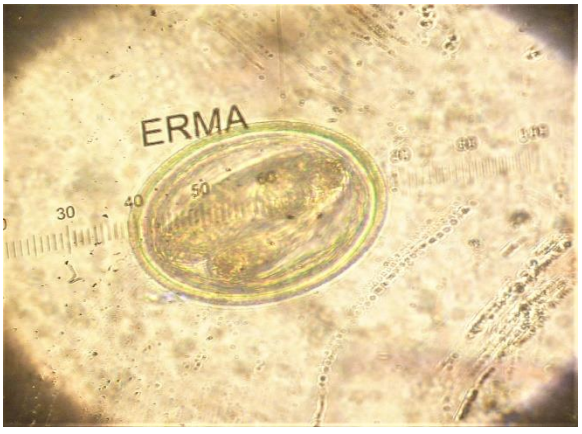

Photo 12: *Strongyloides* sp. egg (71x66 µm)

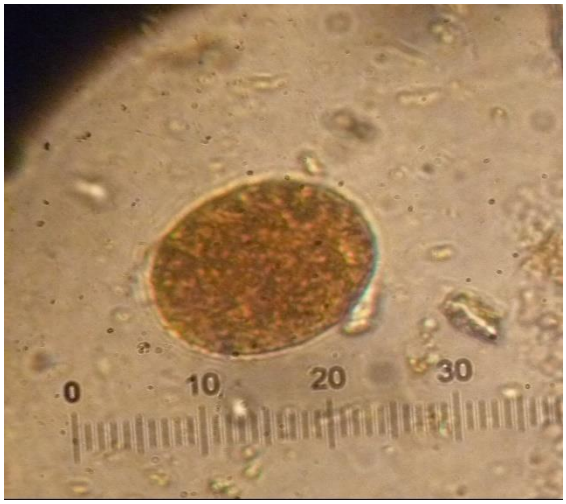

Photo 13: *Codiostomum* sp. egg (38x29 µm)

5. DISCUSSION

The high rate of gastrointestinal parasites may be due to different factors such as stressful life, feeding behavior, constantly keeping birds in the same cage for long period and defecating openly on the ground. The gastrointestinal parasites can be easily transmitted through the mechanical vectors like flea, rats, cockroaches and beetle from one source of infection to another. Altogether 100 faecal samples were examined in the present study, which was carried out to determine the prevalence of gastrointestinal parasites of exotic avian fauna at Central Zoo, Jawalakhel, Lalitpur, Nepal. The prevalence rate (54%) of present study also indicates health of captive exotic avian fauna kept at Central Zoo of Nepal is in risk. The different faecal qualitative methods, namely direct smear method and Concentration techniques (flotation and sedimentation method) used in present study were also used in previous studies (Patel *et al*., 2000; Momin *et al*., 2001; Parsani *et al*., 2003; Goldova *et al*., 2006; Ibrahim *et al*., 2006; Borghare *et al*., 2009; Santilli and Bagliacca, 2011; Prathipa *et al*., 2013 and Ederli *et al*., 2015).

The overall prevalence rate of gastrointestinal parasites in the present study was found to be 54% which was nearly similar to the prevalent rate 48.2% and 48% recorded in the previous study Goldova *et al*., 2006 and Patel *et al*., 2000 respectively. Similarly, the overall prevalence rate of GI parasites in present study was higher than prevalence rate 44.93%, 42%, 41.83%, 27%, and 23% recorded by previous studies (Parasni *et al*., 2003; Reissing *et al*., 2001; Pavloic *et al*., 2003; Papini *et al*., 2012; Edosomwan and Ogbonnia, 2014) respectively. The higher prevalence rate may be due to infection or reinfection (directly or indirectly) of parasites which shows the poor management system of captive avian fauna in zoological garden (Opara *et al*., 2012). Moreover, the prevalence rate of present study was lower than prevalence rate of 88%, 86%, 80%, 71.43%, 68%, 67.7% and 66% recorded by previous studies (Ibrahim *et al*., 2007; Attah *et al*., 2013; Singh and Muhammed 2016; Momin *et al*., 2001; Mir *et al*., 2016; Borgsteede *et al*., 2000; Draycott *et al*., 2013) which indicates more care is given to captive birds in Central Zoo of Nepal as compared to other Zoos. The variation in prevalence rate of GI parasites in birds kept at various Zoological Garden may be due to different number of sample, different methods used, different management practices and sanitary condition, different anthelminthic treatment and season of sample collection.

During this study, one protozoan and four helminth parasites were recorded by using both direct smear and concentration technique. The only single protozoan parasites recorded in this study was *Eimeria* oocysts similar results were also obtained in previous studies (Ibrahim *et al*., 2006; Eslami *et al*., 2007). In the present study, the protozoan prevalence rate 37% was similar to 38% and 31.6% of previous studies (Mir *et al*., 2016; Reissing *et al*., 2001) respectively. The protozoan prevalence rate in present study showed the higher prevalence than 10.40%, 6%, 17.92% and 4.1% of previous studies (Prathipa *et al*., 2013; Varadharajan. A and Kandasamy. A, 2000; Patel *et al*., 2000; Papini *et al*., 2012) respectively.

The high prevalence rate of *Eimeria* sp. in the present study may be due to contamination of drinking water and food by faecal matter of infected captive birds as well as contact with free

foraging birds and poor management system. However, protozoan parasite rate in present study is lowered in comparison to 46% and 85.48% prevalence rate determined by (Singh and Muhamed, 2016; Parsani *et al*., 2003) respectively. The captive birds infected with protozoan parasites such as *Eimeria* sp. generally exhibits weakness, loss of appetite, poor feeding, ruffled feathers and bloody diarrhea which can be diagnosed by post mortem examination (Dingle and Shanawany, 1999). The difference in prevalence rate of coccidian parasites in captive birds reported in various studies might be due to difference in management practices, sanitation, temperature and humidity.

The occurrence of helminth infections in zoo animals that were mainly caused by Ascarids, Trichostrongylidae, Strongylidae, *Capillaria* sp., *Syngamus* sp., *Heterakis* sp and *Strongyloides* sp. (Momin *et al*., 2006; Cordon *et al*., 2009; Lima *et al*., 2016) similar types of parasitic infections were also observed in the present study. Among helminth parasites, four species of nematodes were reported during the study. Among the four species of nematode parasites, the present study confirmed that *Ascaridia* sp. is the most predominant parasites of exotic avian fauna in Central Zoo of Nepal. The decrease in prevalence of helminth parasites in the present study compared with other several studies could be due to better management practices and existence of unfavorable climatic condition or environmental factors that reduce the survival and development of infective larval stage of most nematodes (Thawait *et al*., 2014).

In the present study, the prevalence rate 23% of *Ascaridia* sp. were consistent with 19.5% obtained by previous studies (Attahetal, 2013). The prevalence of present study was higher as compared to the 11.20%, 10.5%, 10%, 7.69%, 6.8%, 4.9% of previous studies (Prathipa *et al*., 2013, Goldova *et al*., 2006; Mir *et al*., 2016; Momin *et al*., 2012; Papini *et al*., 1012; Cordon *et al*., 2009) respectively. This is probably due to the different climatic factors in different Zoological Garden and the possible migration of *Ascaridia* sp. to the liver, trachea and lungs for development also suggest low prevalence rate (Michel, 1974). The prevalence rate of *Ascaridia* sp. in present study was lower as compared to 76.66% and 67% reported in previous studies (Borghare *et al*., 2009; Parsani *et al*., 2003). The higher prevalence rate of *Ascaridia* sp. in previous studies may be due to difference in sample collection method as they have collected samples from adults, squab and examined by postmortem examination and concentration techniques. Management practices and sanitation as well as season also affect the different in prevalence rate. Infection with *Ascaridia galli* in White Rumped Vulture and Slender Billed Vultures at Chitwan district was also reported (Gupta and Pandey, 2007).

Strongyloides sp. infection in the present study encountered the prevalence rate (7%) which was quite higher than 2% obtained in previous study (Prathipa *et al*., 2013). In the present study, prevalence rate of *Strongyloides* sp. was lower than 14% and 20% of previous studies (Mir *et al*., 2016; Singh and Muhammed, 2016). Goldova *et al*., (2006) obtained 2% prevalence rate of *Trichstrongylus* sp. which was lower than 10% prevalence rate obtained in present

study. The prevalence rate of present study was quite similar to 8.25% and 7.69% of previous studies (Reissing *et al*., 2001; Momin *et al*., 2001).

Presenti *et al*., (2015) recorded 79.41% prevalence rate of *Codiostomum* sp. in Ostriches examined by postmorterm examination which is higher than 3% prevalence rate obtained in present study. Ederli *et al*., (2015) also detected the occurrence of *Codiostomum* sp. in the faecal sample from ostrich breeding farm in the state of Rio de Janerio by concentration techniques. Infection with *Codiostomum* sp. in flightless birds such as Emu and Ostriches were also reported (Ederli *et al*., 2014).

Among the infected exotic birds of Central Zoo, order Struthoiniformes show higher prevalence of mixed parasitic infections of *Eimeria* sp., *Ascaridia* sp., *Trichostrongylus* sp., *Strongyloides* sp. and *Codiostomum* sp. in the present study. *Codiostomum* sp. is one of the most pathogenic nematode in flightless birds such as Ostriches and Emus of order Struthoiniformes as described in previous study (Eslami *et al*., 2007) similar parasites *Codiostomum* sp. was also observed in present study. In the order Galliformes, *Ascaridia* sp. revealed more prevalent followed by *Eimeria* sp. similar observation were found in order Galliformes in previous studies (Cordon *et al*., 2009; Santilli and Bagliacca, 2011; Singh and Muhammed, 2016). In the order Passseriformes, *Ascaridia* sp. and *Eimeria* sp. of parasites were revealed during the study and has showed the lower prevalence of GI parasites than other orders.

Altogether 100 faecal samples were examined in exotic avian fauna of Central Zoo, protozoan and nematode infection were found positive in 37 and 33 samples respectively. In general, the protozoan and nematodes having direct life cycle have high prevalence in present study while the parasites such as cestode and trematode having indirect life cycle were not found throughout the study period. This may be due to the absence of intermediate host such as snails. The presence of cestodes and trematodes has been recorded in different literature during literature review. In present study, there was absence of cestodes and trematodes which was also absence in previous studies (Parsani *et al*., 2003; Mir *et al*., 2016; Otegbade and Morenikeji, 2012). Most of the captive exotic birds of Central Zoo has revealed mixed infection of *Eimeria* sp. and *Ascaridia* sp. and *Strongylus* sp. Similar type of mixed infection were also observed in previous studies (Parsani *et al*., 2003; Ibrahim *et al*., 2006; Teixeira *et al*., 2012 and Muhammed, 2016).

In the present study area, all the assistant Zookeeper do not have proper knowledge about parasite and parasitic disease. Stressful life, feeding behavior, anthelminthic treatment were done only for captive birds not for surrounding foraging birds, constantly keeping birds on the same cage for long periods and irregularly cleaning of feeding apparatus can also increase the rate of parasitic infection to captive birds. The foraging birds such as Pigeons, Crows, Sparrows and Rats were easily entered in some cages and feeds on food and water supplied to captive birds of Central Zoo then defecating openly on the ground that cause contamination of food and water. By consuming the contaminated water and food with infective stage of parasites such as cysts, eggs and larval forms, these captive birds present at Central Zoo can also be

infected different gastrointestinal parasites. This may be the reason why 54 samples were found to be infected with one or more types of gastrointestinal parasite which was recorded throughout the present study period.

The existing infection can be controlled by adopting suitable anthelminthic therapy and management practices, but the possibility of reappearance of parasitic infection is always there due to stress and also due to closer proximity birds in a very small cages. The possibility of transmission of zoonotic infections also exist among the captive birds and the assistant zookeeper therefore, requires investigations.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Parasites are the neglected topic but study of parasite and parasitic disease play important role in the conservation of captive birds in zoological gardens. High parasitic load may cause growth retarded, anemia and hemorrhagic condition causing serious death in birds.

Overall, present study demonstrate the prevalence of gastrointestinal parasitic infection in captive exotic avian fauna in Central Zoo of Nepal was 54% with the higher prevalence rate of *Eimeria* sp. using direct smear method and concentration techniques. During faecal examination, five number of parasitic genera were observed. Among them, one protozoa: Eimeria sp. and four helminths: *Ascaridia* sp., *Trichostrongylus* sp., *Strongyloides* sp. and *Codiostomum* sp. Among helminths, the *Ascaridia* sp. showed the highest prevalence rate (23%) followed by *Trichostrongylus* sp., (10%) *Strongyloides* sp. (7%) and *Codiostomum* sp. (3%). All these recorded helminths were nematodes but trematodes and cestodes were not recorded from this study. Among 16 exotic birds present at Central Zoo of Nepal, the highest prevalence of GI parasites were recorded in African Ostriches, African Grey Parrot, Guinea fowl, Reeves's Pheasant and Salmon Crested Cockatoo revealed the highest prevalence (100%) of gastrointestinal parasites infection followed by Emus (75%), Blue and Yellow Macaws (66.66%), Cocatiels (60%), Golden Pheasants (58.82%). Budgerigers show higher prevalence of gastrointestinal parasites than Silver Pheasants. Lady Amherst Pheasants and Peach faced Lovebirds show equal prevalence (40%). Java sparrow, Sulpher-Crested Cockatoo and White Crested Cockatoos also show equal prevalence of (33%). The study confirmed that the captive birds kept in Central Zoo were found to be susceptible and infected by various gastrointestinal parasites.

Stastically, the difference in prevalence of helminthes and protozoan parasites was found to be insignificant but the difference in prevalence of single and mixed infection was found to be significant. The present study shows captive exotic birds at Central Zoo were more infected with single infection than mixed infection. The current management practices adopted in the present study area was good. In addition, by controlling the foraging birds that enter inside cages help to reduce the prevalence of endoparasites of captive birds. Therefore, effective deworming of foreiging birds can be done to upgrade the health status of captive exotic birds at Central Zoo of Nepal. The prevalence rate of parasitic infection in the present study show an alarming situation of health status of captive exotic birds in the study area. This finding might be considered while designing control strategies of gastrointestinal parasites in captive at Central Zoo of Nepal.

6.2 Recommendations

- Further study or molecular level identification of parasites should be done.
- Seasonal wise study of gastrointestinal parasites of zoo birds should be done.
- Evaluation of comparative efficacy of different anthelminthic against GI parasites can be conducted.

- Comparative study of GI parasite between captive and foraging birds at Central Zoo are recommended.
- Current management practices should be improved regarding feeding and sanitation.
- Overcrowding of captive birds in different cages should be checked.

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APPENDIX 1

QUESTIONNAIRE

GI- parasites of exotic birds in Central Zoo, Jawalakhel, Lalitpur, Nepal.

Name…… Educational level……..

Age ……. Designation……..

Gender……. Section…….

Service year……

1) Do you know about exotic birds?
   A) Yes B) No
2) What is the initial action taken soon after the exotic birds bringing inside the zoo?
3) How many species of exotic birds present in the Zoo?
4) Can you distinguish their age?
   If yes, which age of birds were more infected to parasites?
5) From where feeding materials are supplied to captive birds?
6) At what time do you provide food to them?
   A) Morning B) Day C) Evening
7) What is the source of water given to the birds?
   A) Tap water B) Well water C) Tanker water D) Others
8) Is feeding by visitors permitted?
   A) Yes B) No
9) Are the cages of capive birds cleaned every day? If at what time?
   A) Yes B) No
10) Which chemical disinfectant are used to keep the cage clean?
11) Where did the faecal waste disposal?
12) Is there proper management of drainage?
   A) Yes B) No
13) How about hygiene of kitchen and safe behavior of animal keeper?
14) Is the health of those birds regularly monitored?
   A) Yes B) No
15) What kind of disease they suffer more frequently?
16) What is done in emergency case when captive birds get ill?
17) What are the management practices done to dead birds?
   A) Buried B) Postmortem (C) Others
18) Did you provide antibiotics vaccine to exotic birds?
19) How the population of captive birds are managed?
20) Are anthelminthic drugs regularly provided to both captive and foraging birds?
   If yes, how many times it is provided in a year?
21) Do you found deworming programme is effective?